Ultrahigh Productivity Photobioreactors for Algal Biofuel Production

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Abstract

Algal biodiesel is a biodegradable and sustainable alternative to traditional petroleum fuels. Algal biodiesel is synthesised from algal lipids via transesterification and has many desirable physical properties for fuel use. Current photobioreactors are inefficient. This thesis looks to increase efficiency and reduce energetic running costs. This was undertaken by the design, construction and trialling of an LED photobioreactor. The controlled growth of the algae, specifically *Chlorella emersonii*, using pulsed monochromatic or bi-chromatic light conditions with comparison to continuous white light to improve light economy is explored in this thesis. The prediction of biodiesel profile from the growth conditions is also investigated for *Chlorella emersonii*.

Chapter 1 is a general introduction to the area of algal biodiesel. This introductory chapter reviews the current literature regarding microalgae growth conditions and control, processing microalgae to produce biodiesel and photobioreactor designs for the controlled growth of algae. The known effects of different light sources and types on algal growth are also reviewed.

Chapter 2 concerns the pulsing-LED vertical airlift photobioreactor design, construction and testing, including an overview of the system constructed and the process of design to combat specific issues. Results from the testing of the photobioreactor are reported in this chapter which include analysis of the resultant fatty acid methyl ester (FAME) profile of algae grown under various pulsed mono-chromatic and bi-chromatic light conditions and the comparison to continuous white light. This chapter draws together the hypotheses and stand-alone observations reported in the current literature allowing direct comparisons for different light conditions and conclusions to be reported which include the effect on resultant FAME profile and not just lipid percentage.

Chapter 3 explores the effect of environmental factors on the fatty acid methyl ester composition of the algal biodiesel. This chapter describes the effect of carbon dioxide, nitrate, phosphate and iron levels, length of culture and the effect of supplementary carbon sources on *Chlorella emersonii* growth and resultant FAME composition. The result of synergetic effects of nutrient levels and length of algal cultivation are analysed in addition to the stage of algal growth and its impact on FAME profile.

Chapter 4 details the procedures used for the growth of algae, the production of the algal biodiesel and the development of techniques used for analysis of the resultant biodiesel. The techniques and conditions employed for the growth of the algae as well as the extraction and transesterification of the algal lipids are explained.

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1 Introduction

1.1 Preamble

Environmental change, such as carbon dioxide level increases, and global warming in addition to current difficulties in fuel supply demand a cohesive and twenty-first century solution. The need for alternative fuel sources has been well publicised by media and science. Biodiesel should be at the forefront of the switch over to green energy as it requires little, if any, modification of diesel engines as well as requiring minimal integration into the fuel supply network. Algae are an excellent candidate for biodiesel production due to their high bio-productive rate and the economic potential for valuable product harvesting. Algae have also been proven to sequester carbon dioxide and shown to reduce heavy metal contamination in waste water.[1] The triglycerides present in algae can be extracted and transesterified into biodiesel for use as liquid transportation fuel.[2]

This introduction will review relevant aspects of biodiesel production, algae cultivation and photobioreactor design including the economical, energy and environmental outlooks for algal biofuel.

1.2 Biodiesel

Biodiesel is the transesterification product, fatty acid alkyl ester, of triglycerides from land crop oils, animal fats and more recently algal oils. As shown in Figure 1:1 the transesterification reaction requires an alcohol and a catalyst (see section 1.2.10).[3]

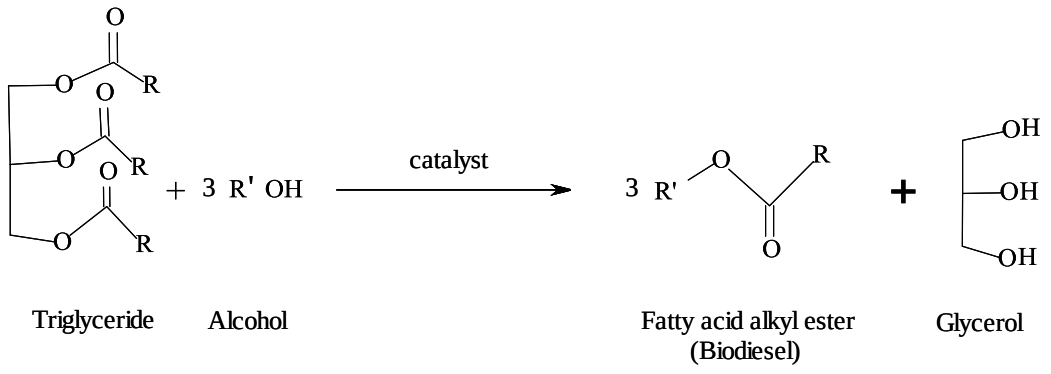


Figure 1:1 – Transesterification reaction overview

1.2.1 The requirement for biofuels as a transitional technology

Two thirds of the whole world's total energy consumption remains sourced from traditional fossil fuels; hence the urgency due to diminishing fossil fuels to develop other means of sustainable energy production.[4] The increase in demand for petroleum, particularly from emerging markets such as Asia, is increasing the requirement to expand renewable fuels, specifically liquid fuels like biodiesel.[2] The transportation industries, including aviation and shipping rely heavily on liquid fuel derived from petroleum.[5] Biofuels are needed to improve energy security (see section 1.2.7), climate change and rural development.[6] Other issues which biofuels can address are increasing global population, mounting waste, depleting resources and increased environmental pollution.[7]

Biodiesel can have comparable energy content and similar physical properties, such as cetane number and cold pour point, to petroleum diesel thus making it a desirable target for biofuel production. The use of transesterified plant oil as a fuel is credited to E. Duffy and J. Patrick in 1853, whilst R. Diesel showcased the first engine running from vegetable oil in 1900 at the International Exhibition, Paris.[8] The three main targets for any biofuel are to have (i) high energy density with regards to mass and volume; (ii) to be producible at yields around the stoichiometric maximum for the chosen biomass starting material; and (iii) to be compatible with the existing fuel distribution infrastructure.[9]

Mass changeover to electrical and hydrogen powered terrestrial transportation is expected to begin within the next couple of decades; this widespread uptake can then be more fully developed.[4-5] Biofuels, particularly biodiesel, can fit this energy gap and help ease predominantly industrialised countries into a future without dependence on fossil fuels. Biofuels are required to have beneficial properties, such as high density liquid energy, fuel security and easily biodegradable products (see section 1.2.4).[2, 10] It is sought-after that the biofuel has a lower carbon footprint than that of the fossil fuel it replaces and that the biofuel is suitable for the use it is being employed for. It may also be considered that subsidies may be required for renewable biofuels to be commercially available. It is desirable that the production of high value co-products alongside biofuel will allow biofuel, especially biodiesel to become economically viable. However, when high value products are created on the large scale required for fuel production they may lose their high-value status due to market saturation.[11]

From a fuel security perspective the use of biofuels is advantageous due to the relief of dependency of countries upon other world regions for fuel. The growth of fuel crops close to production plants will minimise infrastructure and transportation costs.[12] Biodiesel, due to its liquid nature, can be transported through pipelines which reduce distribution costs when compared to inefficient methods such as road and rail.[12] Different fuels have different energy outputs, No. 2 diesel has a volumetric energy density of 38.3 MJ L^{-1} whilst biodiesel produced from virgin canola oil has an output of 35.7 MJ L^{-1}.[9] Biodiesel from plants has 90 % energy of that of petroleum diesel and more energy than ethanol fuels. Biodiesel derived from soybean oil has 93 % net energy return whereas ethanol from maize has just 25 % net energy return. The success of soy-biodiesel over maize-ethanol is due to lower operating costs of soy and the higher energy content in biodiesel compared to ethanol.[13] Rapeseed, which is predominantly used for biodiesel production in Europe, is easier to grow in suboptimal conditions and has a higher oil seed content (30 – 40 % *cf*. 20 % in soybean).[4]

1.2.2 Fatty acid methyl ester (FAME) profiles and fuel properties of biodiesel

The fuel properties of biodiesel depend upon the profile composition of fatty acid methyl esters; including fatty acid chain length, free fatty acid percentage and degrees of unsaturation.[14] Most commercial biodiesel is 5 – 8 % less efficient than conventional petroleum diesel however biodiesel has reduced emissions, apart from an increase in NO_x emissions.[15] Commonly in Europe diesel sold contains ~5 % biodiesel and where pure biodiesel is sold it usually contains 1 % petroleum diesel to inhibit mould growth.[16] The nomenclature used to label fatty acid methyl esters is in the form Cx(y) where the x represents the length of the ethyl chain and the y indicates the number of double bonds within that chain (Figure 1:2). Additional information to label the position of double bonds in the carbon chain are noted by the use of 'n' for the total number of carbons from the distal (last) double bond to the terminal methyl. The position of any further double bonds present are usually determined by adding 3, in the case of conjugated double bonds there are just 2 carbons between each bond.

The analysis of fatty acid composition of the biodiesel obtained for suitability and compliance with ASTM D6751 and EN 14214 biodiesel standards can be carried out, including viscosity, oxidative stability, and fuel density.[3] Petroleum diesel can be

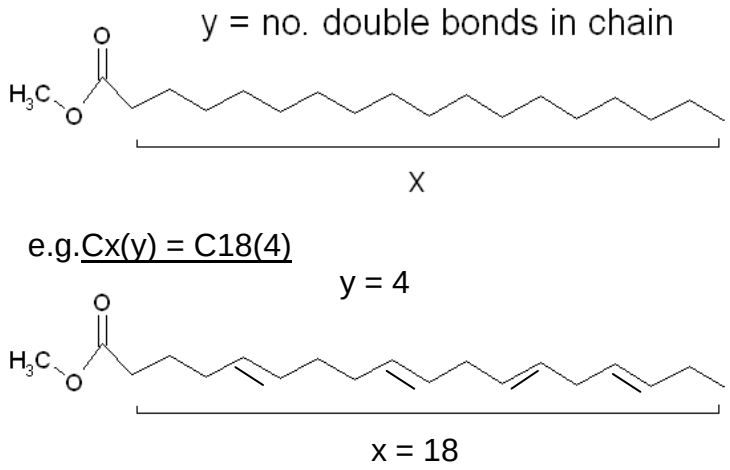


Figure 1:2 – Nomenclature for fatty acid methyl esters

substituted for biodiesel when they have similar ignition qualities *i.e.* cetane number, similar heat of combustion, cloud point, pour point, oxidative stability, kinematic viscosity and lubricity (Table 1:1). Biodiesel is made up of 15.2 % aliphatic and 84.7 % olefinic compounds whereas diesel contains 67.4 % aliphatic, just 3.4 % olefinic compounds. Diesel also contains 20.1 % aromatic and 9.1 % naphthene compounds, and its exhaust gas contains over 300 compounds.[8, 17]

Table 1:1 – Physical properties of algal biodiesel presented with European standards for diesel and biodiesel[3, 18]

Properties	**Algae biodiesel**	**EN 590 (Diesel)**	**EN 14214 (Biodiesel)**
Fuel density (g cm^{-3})	0.864	0.820-0.845	0.860-0.900
Kinematic viscosity (mm^2s^{-1} @ 40 °C)	5.2	2.0-4.5	3.5-5.0
Flash point (°C)	115	>55	120
Cetane number	57	51	>51
Cold filter plugging point (°C)	-11		-5
Acid value (mg KOH g^{-1})	0.374	<0.5	<0.5

Chain length and saturation of the fatty acid alkyl esters (FAAEs) depends on the algae species (or vegetable oil) used and the growing conditions (Figure 1:3).[1, 3] There will be a variety of lipids present in the algae oil, including triglycerides, diglycerides, monoglycerides, glycerol, phospholipids and glycolipids, forming an assortment of FAAEs on transesterification. This affects the fatty acid profile and performance characteristics of

the biodiesel.[19] If there are high ratios of unsaturated fatty acids present, like C18(2) or C18(3), lower cetane numbers and high iodine values prevail.[20] Cetane numbers are an arbitrary measurement used to scale the combustion quality of diesel fuel; the higher the cetane number the quicker the fuel will ignite, the more effectively it will combust and there will be fewer emissions released.[20a] Iodine number is used to determine the degree of unsaturation within the fuel; this can indicate how stable a fuel is.

The difference in FAME composition between terrestrial crops and algae are many and various depending on the type of terrestrial crop and the species of algae as well as depending upon the growth conditions of either. However as can be seen in Figure 1:3 there is a predominance of C16 and C18 derived FAMEs in terrestrial crops with degrees of unsaturation of 1, 2 or 3. On the contrary for some algae species higher degrees of unsaturation can occur, such as C16(4), C18(4) and C20(5) as well as both shorter and longer chains being common.[20b, 21] The higher degrees of unsaturation can be undesirable in conventional biodiesel due to uncertainty about stability; however sufficient anti-oxidant can counteract poor stability. Accelerated oxidation tests were carried out by R. Jenkins, C. Chuck and J. Lowe at the Chemistry Department in the *University of Bath*. These tests showed the breakdown of commercial B100 from *British Petroleum* occurred in around 8 hours whereas biodiesel produced from *Chorella emersonii* maintained integrity even after 72 hours of testing. The improved oxidative stability was attributed to the natural antioxidants present in the algae fuel. Lower stress conditions, such as low temperature and light conditions often yield higher unsaturated fatty acids whilst increased temperatures and light conditions generally lead to more saturated fatty acids being formed.[8, 20b, 22]

1.2.3 Issues with first generation biofuels

Conventional biofuels, such as those produced from starch, sugar or vegetable oil are known as first generation biofuels; over-production of first generation fuels will impact on food supply and biodiversity.[23] Advanced or second generation biofuels, are those produced from sustainable feedstocks, for example cellulose, as well as other biofuels synthesised from biomass which is either industrial waste or a non-food crop.[23a] Third generation biofuels are those which have been genetically modified in some way, and are carbon neutral, whilst fourth generation fuels have the additional advantage of being carbon negative.[23b]

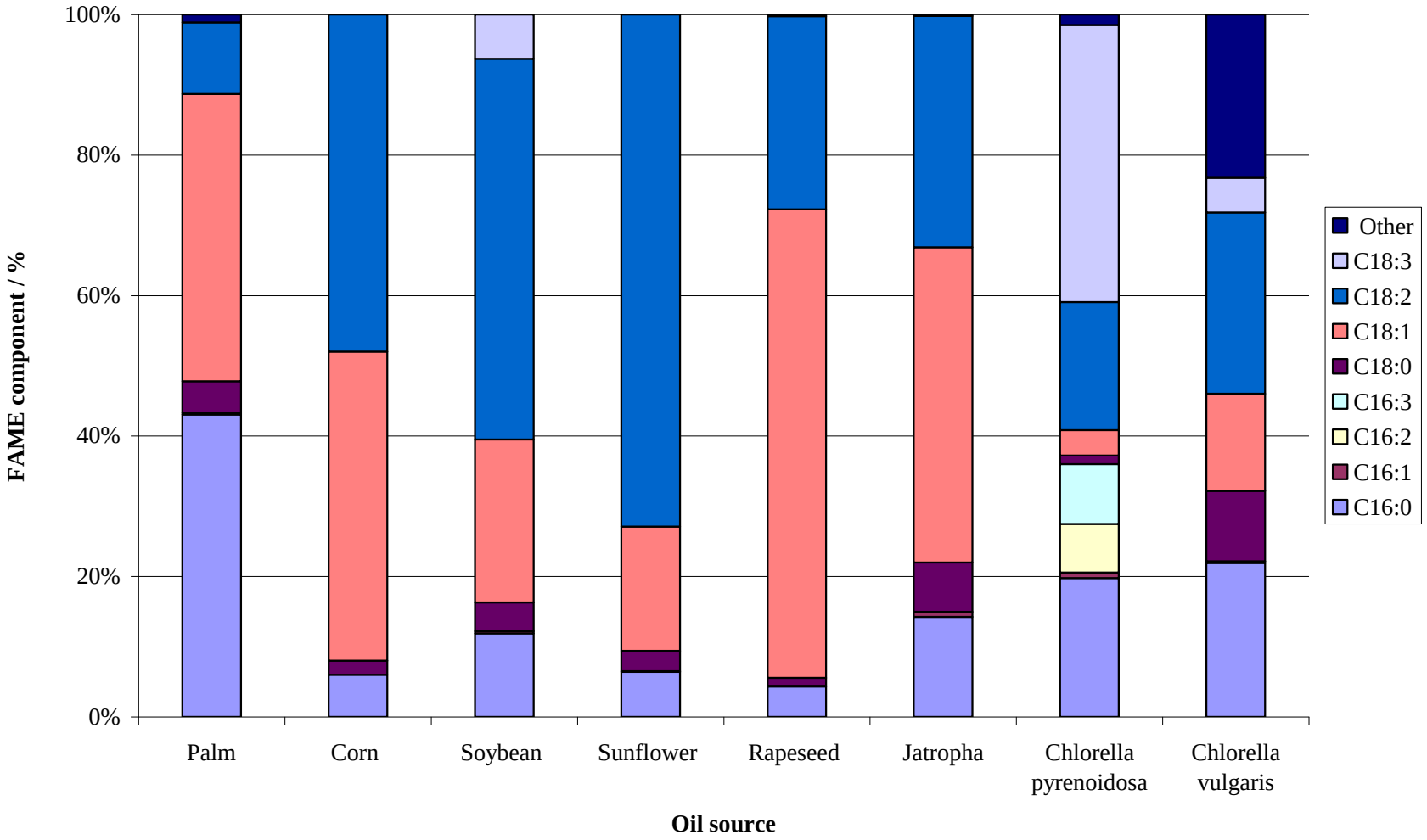


Figure 1:3 – Biodiesel FAME components from various oil sources[21a, 26]

The drawbacks of using first generation biodiesel compared to petroleum diesel include dependency on growth seasons of the crops utilised and deforestation of rainforest for fuel crops. For the displacement of fossil fuel, particularly petrol and diesel the successful forerunners are ethanol derived from sugar cane and biodiesel from palm oil. For both of these fuels there are specific geographical limitations of the economic feasibility.[24] Both sugar cane cultivation in South America and palm nut cultivation in South East Asia are responsible for the overall reduction in rainforest habitats.[25] The uptake of biofuels is expected to increase above the assumed limit of first generation biofuel production, 4 – 5 $x10^6$ barrels per day, in the next 15 years.[6]

The disadvantages of biodiesel over petroleum diesel lie in its engine interactions, where fuel injection has caused concern potentially due to its higher viscosity than petroleum diesel. This increased viscosity causes higher surface stress of the biodiesel on the engine.[8] Another potential problem is the lowered crystallisation temperature, thus B100 can require preheating of fuel prior to it entering the fuel pump.[8] Oxidation stability is, perhaps, the major concern for fuel manufacturers due to the increase in oxygen within the biodiesel. Another disadvantage of the currently used biofuels is the use of corrosive and toxic sodium methoxide as a catalyst in the manufacture of the fuel (see section 1.2.10).[27] Catalyst development, especially heterogeneous catalyst development, for the production of biodiesel from biofuel sources and particularly algal lipids should remove issues surrounding the corrosive nature of catalysts in the engine.[27]

Environmental issues with methanol and/or ethanol used in the production of biodiesel could be mitigated by the production of bioethanol from the carbohydrate fraction of the spent algae.[27] Other issues are those surrounding the use of food sources as fuel which could cause shortages of these foodstuffs.[28]

1.2.4 Potential of algal biofuels

Algae can be used as a fuel directly[29], converted to bio-alcohol[9, 30], or bio-oil[31] and cultured for the collection of biogas (hydrogen).[32] The comprehensive use of the different constituents of algae, *e.g.* lipid, carbohydrate and protein, will increase its green potential.[13] Algae can also be used to form electricity[33], for the conversion of algae lipids, via hydrogenation or transesterification to biodiesel.[34] The use of algal biodiesel is environmentally preferable to petroleum diesel due to the 30 % reduction in sulfur oxide

emissions and 10 % decrease in carbon monoxide emissions. This lowering of emissions leads to a reduction in total air toxicity of up to 90 %. Biodiesel has a superior flash point and biodegradability than petroleum diesel.[34]

Algae can contain a higher weight percentage of lipid than terrestrial crops (up to 80 %) which can be transesterified into biodiesel.[33] Photosynthesis is the process by which algae and terrestrial crops, as well as the precursors to petroleum fuel, obtained their energy (see section 1.5.10).[35] Algae intrinsically offer a greater flux tolerance than other plants as well as increased photosynthetic efficiency due to minimal alternative plant functions and focussed modular growth.[36] Algae have limited nutritional requirements and can be exposed to temporal and spectral irradiation distributions of varying intensities which, despite not predominantly occurring in nature, are optimal for increased bio-productivity.[36a, 37]

In the case of spillages, algal biodiesel breaks down relatively quickly in the environment causing minimal setbacks to the ecosystem in which such accidents occurred.[38] Algae have, furthermore, shown the potential to degrade petroleum oils which are notorious for their detrimental impact on the environment when spillages occur. At least nine cyanobacteria, five green algae, two diatoms, one brown alga and one red alga have been indicated to oxidise naphthalene.[38-39]

The analyses for five of the main contenders for future biodiesel production have been analysed, by Dinh *et al*..[18] The environmental, economic, safety and fuel performance indicators are shown in Table 1:2. Algae have a very positive sustainability profile, particularly in terms of greenhouse gas emissions, water usage and land mass required as well as good safety indicators. The fuel performance of algal biodiesel is comparable to the other feedstock biodiesel.[18]

Commercialisation of algae cultivation would enable the harvesting of metabolic products for consumption by marine and terrestrial organisms.[40] The use of genetic modification, metabolic engineering and directed evolution can all enhance lipid production and high value product yield, to ensure commercial viability.[41] The growth of algae for fuel and high value products alongside carbon mitigation or wastewater treatment highlights its versatility and potential for sustainable and economic profit (see sections 1.2.8 and 1.2.9).

Table 1:2 – Sustainability parameters for the feedstocks - jatropha, algae, palm oil, rapeseed and soybean * to meet all transport fuel needs of USA, ** algae with 30% oil in biomass[18] (GHG – greenhouse gases)

		Environmental indicators		**Economic indicator**		**Safety indicator**		**Fuel performance indicators**	
Biodiesel source	GHG emissions (gCO_2-eq/MJ)	Water usage ($g/m^3/day$)	Land area required* (M ha)	Total production costs ($ L^{-1} biodiesel)	Optimum molar ratio (MeOH : oil)	Flash point (°C)	Cetane number	Cloud point (°C)	Carbon residue (%wt)
Jatropha	56.7	3000	280	0.682	5.5	175	51	13	0.02
Algae	3	16	9**	0.619	3	196	57	2	0.03
Palm oil	138.7	5500	90	0.661	7.5	164	62	13	0.02
Rapeseed	78.1	1370	446	0.729	8	180	56	-2	1.1
Soybean	90.7	530	1188	0.571	6	178	45	1	1.74

Organisms such as plants and algae, which use predominantly light and carbon dioxide to grow, are especially environmentally interesting due to their potential to mitigate carbon dioxide.[22a] Microorganisms, such as algae, have advantages over terrestrial crops due their potential high growth rates and high biomass yield as well as not requiring traditional crop growing area and can be grown on marginalised land.[13, 22a, 26a, 28] Algae have little or no competition or current value within the food market, particularly in the West.[42] Algae have minimal impact on freshwater supply since many species are capable of growing in saline or brackish water and algae require less water than oil-seeds.[2, 13, 42-43] Wastewater is a secondary effluent with low biochemical and chemical oxygen demand, yet maintaining high inorganic nitrogen and phosphorous.[44] The uptake of metals by algae can be utilised to purify contaminated wastewater[45] and the use of salt water for cooling can further reduce the impact of algae on freshwater supplies.[46] Microalgae has species-dependent increased efficacy for removing metal species from solution compared to fungal or bacterial biomass growth; algal surface area and cell wall composition affect metal binding efficiency and affinity.[47]

1.2.5 Problems with algal biofuels

Issues such as ambient temperature maintenance and adequate light are prevalent for the health of algal cultures, since natural climates are subject to variance (see section 1.5.10). It is also known that algae which grows and thrives in open ponds is often high in carbohydrates and proteins.[2] The growth of mass algal biomass in a controlled and predictable manner necessitates further research, for consistent outputs of lipid with acceptable and desirable FAME profiles. The contamination of algal cultivations is also a sizeable problem, which is currently being combated with a combination of specific reactor design and algal species which grow under adverse conditions (see section 1.5.1). Extraction of lipids from algal cells can be challenging and requires further development (see section 1.4.1).

The current three hurdles for economical and sustainable algae biofuel usage are: (i) cost; (ii) identification of a strain with high enough lipid content and fast growth rate which is easy to harvest; and (iii) effective design of a bioreactor.[2]

1.2.6 Commercial ventures in algal biofuels

Algae can grow in closed bioreactors in the most varied and severest of climates with challenging land topography and geology therefore algae biofuel is relevant to a worldwide market.[48] There are currently between 40 and 50 algal biofuel companies, spin-off companies and government funded initiatives based all over the world, from Marlborough, New Zealand to Capital Federal, Argentina and from Scotland to Honolulu, USA. Some of these companies suffered under the economic changes around 2008-2011 and so do no longer exist, however each month more and more are starting up with varied and specific targets and markets. Many companies are aiming to utilise algae from carbon capture, mitigation or recycling and cleaning of wastewater, whereas others are looking to the fish food market or energy production. A number of the companies are targeting bioengineering and genetic manipulation to meet their ambitious objectives, such as zero emission fuel or production of plastics, pharmaceuticals and cosmetics (See Appendix 1).

1.2.7 Policies and government goals

Geopolitical issues and policy barriers require consideration as well as the ecological and environmental implications of using algae as a source of fuel, and the supply, storage, technological, technical, economical and safety considerations. Due to 66 % of the world's petroleum fuel reserves lying in the Middle East there have been certain geographical and political implications for its distribution.[49] The production of renewable energy has the potential to be more evenly distributed across the world than fossil or nuclear fuels.[50] Policy hurdles to be overcome include reallocation of land use to fuel production. The use of internally sourced biofuel enables countries to reduce their dependence on imported petroleum fuels and build local economies.[51]

The European Union intended to replace 5.75 % of transportation fuel with biofuels by 2010, this has been achieved for diesel by the substitution of rapeseed biodiesel; the goal for 2020 is a 10 % replacement.[46, 52] Brazil is a well-known leader in the development and implementation of biofuel use. In Brazil 25 % of petrol has been replaced by bioethanol produced from sugar cane since 2003. Brazil has exceeded their 25 % target for petrol replacement due to the competitiveness of bioethanol production. Brazil's target for 2013 is a 5 % replacement of diesel with biodiesel from soybean, castor and palm oil.[46] Specific and significant pledges have been made by emerging economies such as India (e.g. 10 %

bioethanol by 2008) have also made regarding the uptake of biofuel into their transport economy, whilst USA and Canadian pledges have been more moderate.[46]

1.2.8 Economical cost of algal biofuel

Algae-derived biodiesel is currently 4 – 10 times more expensive to produce than petroleum-derived fuel and some other types of biodiesel.[22a] Nevertheless algal biodiesel has a small ecological footprint as a smaller growth area is required and a reduced amount of water is used in closed growth systems compared to terrestrial crops.[22a] The cost of algal biodiesel is the major hurdle for its wide scale implementation.[53] For algal biodiesel to be realised at a commercial scale the investment costs are required to be below €15) per m^2 (equivalent to \$19.80 per m^2 – Nov 2011).[54] Whilst algal biodiesel predicted costs vary dramatically, Dermibas *et al.* and Chisti[22a, 50] estimate the cost of algae oil at \$1.40 and \$1.81 per litre for algae grown in photobioreactors and raceway ponds respectively.[22a] Due to economy of scale-up it is hypothesised that if the photobioreactor and raceway pond increase in capacity to 10,000 tonnes the actual cost of oil would be \$0.47 and \$0.60 per litre.[22a] A paper by Chisti[22a] suggests that to be competitive with petroleum diesel the cost of algal oil should be less than \$0.48 per litre; hence photobioreactor algae growth has potential on a large scale to compete with petroleum biodiesel.

The Bloomberg New Energy Finance Magazine[55] reports that the cost of producing algal biofuel from photosynthetic pathways in 2010 was currently around \$7 per litre. The predictions within the article made by researchers were that the actual cost could fall to \$4.50-\$6.50 per litre for photobioreactor grown algal biofuel and \$2.00-4.00 per litre for open pond grown fuel.[55] There is a requirement for competitive, clean and secure supplies of transportation fuels.[22b] Over the coming years fluctuating oil prices are expected to continue and an increase in oil costs of 60 % (by 2030) due to supply and demand issues.[22b] Another contributory factor for oil price increase is competition for resources from emerging transportation industries in India and China.[22b] It has been postulated that if algae biomass can be produced at €0.50/kg (equivalent to \$0.66/kg – Nov 2011) it will be possible to produce biodiesel from algae at a competitive rate.[54] In 2003 the cost for petroleum diesel was \$0.35 l^{-1} whilst biodiesel from soybean in the USA was \$0.5 l^{-1}.[53] Cost of biodiesel is perhaps the major reason for low uptake of biodiesel fuel from any source. Currently feedstock costs account for more than 70 % of the cost of biodiesel production.[53] Evidently algal biodiesel is not, at the moment, economically competitive

with petroleum diesel; however with adequate reductions in capital investment feasibility studies have highlighted the required decrease in production costs is possible.[54]

Other costs to consider for biofuel are the potential impact on employment, income, food and poverty levels in developing countries particularly in rural areas or those with a history of small-scale farming and agriculture.[46] Algal biofuel technologies and local industries can work symbiotically to create local wealth and employment without destroying biodiversity.

Europe's suggestion is that there will be sufficient developments in the algal biofuel market to have an attractive alternative fuel product in the next ten years.[54] The American fuel industry is more positive; predicting that there will be progress to fully commercialise algal oil in the next 4 – 5 years.[56]

ExxonMobil have invested $ 600 million over 5 – 6 years to engineer algal cells which secrete hydrocarbons and lipids into solution in a pure form. Bioreactors and ponds are being used in San Diego, USA to cultivate naturally occurring algae in a focus for project scale up; it is projected that billions of $'s are required for research and development programme to be economically scaled up.[57]

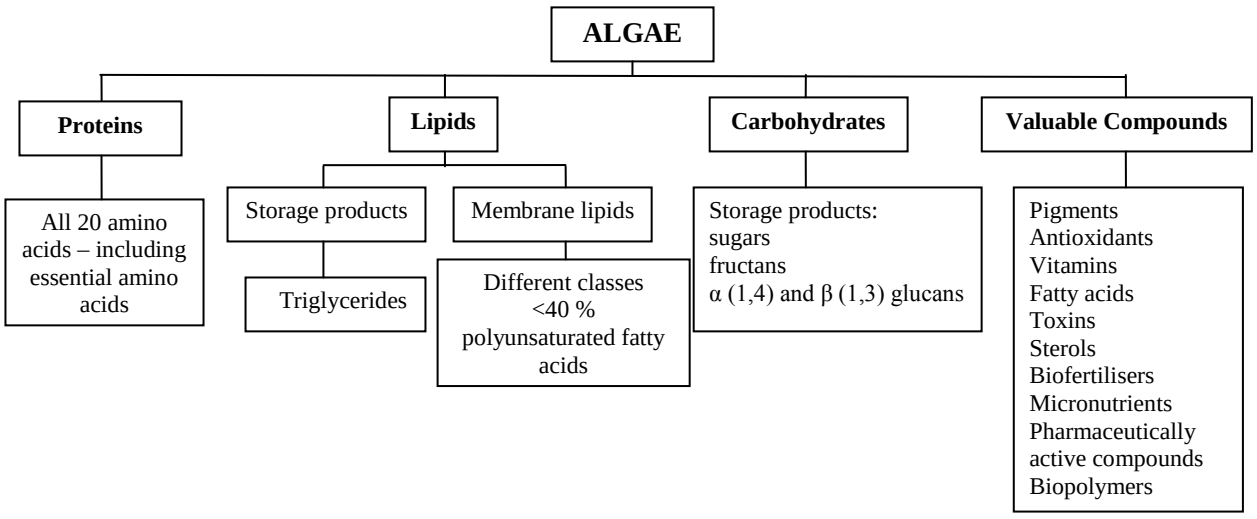


Figure 1:4 – Algae components and uses[31a, 36b, 37, 59a, 60-64]

Integrated systems could be developed to improve the economics of biodiesel production from algae. After the extraction of lipids for biodiesel production the residual biomass can

be anaerobically digested to produce methane.[33, 58] After transesterification the side product, glycerol, could undergo either pyrolysis resulting in syngas, methane and ethane or steam-reforming to form hydrogen.[28, 58-59] Algae also contain many different and varied compounds which can be utilised in a number of ways (Figure 1:4). Algae contain compounds, as shown in Figure 1:4, for general nutrition[36b, 60], pharmaceuticals and fine chemicals[31a, 61], lipids[62], colorants[63] and polymers[31a].

1.2.9 Energy costs and considerations

The energy advantage of using algae for biodiesel production is also related to the other beneficial tasks the photobioreactor is performing, for example, sequestration of carbon dioxide from waste gases[37], removal of contaminants from wastewater[64c, d]and harvesting of high value products.[14, 50] Extraction of the highest possible energy from algae will lead to higher levels of efficiency and sustainability, as well as giving higher economic feasibility.[59a] Bioremediation of wastewater and scrubbing of flue gases are algal technologies which can be retrofitted to existing factories and power stations to reduce their environmental impact.[65] Traditional thermal power stations which produce electricity emit on average 13 % of their total flue gas emissions as carbon dioxide.[50] The biochemical fixation of carbon into new biomass such as algae has the potential to improve air quality, dramatically reduce emissions trading and carbon dioxide certification costs for power-hungry industries.[66] Algae require around half of their biomass weight of carbon dioxide to grow[66]; other authors report that 1 kg dry algae biomass utilises up to 1.83 kg carbon dioxide.[22a, 67] Airlift photobioreactors in series have been reported to minimise carbon dioxide losses compared to sequential bubble column reactors.[68] The airlift photobioreactors sequestered up to 52.5 % of carbon dioxide from flue gas simulated conditions with 15 % carbon dioxide containing air.[68] It has been shown that algae can increase growth rate due to higher carbon dioxide concentrations which results in more carbon dioxide being captured by photosynthesis.[4, 69]

Overall the most energetically intensive steps in the algae biodiesel production process are those involved in lipid extraction from the dry algae mass, attributing around 4 MJ of energy cost for each MJ of algal biodiesel produced.[70] Khoo *et al.* reported life cycle analysis assessments for algal biodiesel production using a hypothetical integrated photobioreactor-raceway pond in Singapore.[70] Khoo *et al.* reported that "Based on a functional unit of 1 MJ biofuel, the total energy demands are 4.44 MJ with 13% from

biomass production, 85% from lipid extraction, and 2% from biodiesel production.".[70] Stephenson *et al.* described the hypothetical cultivation of *Chlorella vulgaris* using a raceway pond and an air-lift bioreactor in series with downstream processing.[71] The result of life-cycle analysis of raceway pond algal growth was a reduction of 80 % of global warming potential, when compared with petroleum fuel. The air-lift bioreactor required significantly more energy to be produced than the fossil derived fuel. Both of these simulations were dependent upon an annual lipid productivity of 40 tons ha^{-1} which is, as yet, unrealised in either system.[71] Other sources report that the extraction of lipids and dewatering stages of algal biodiesel production account for up to 90 % of the total energy requirements.[33] Evidently current photobioreactors are inefficient; consequently this thesis aims to increase the efficiency of photobioreactors and reduce the energy costs associated with algal biodiesel.

Larger reactors which require lower mixing costs incur higher initial energy costs due to more materials being used for construction. Whilst rapid mixing can allow the algae cells to experience the same amount of time exposed to sufficiently high light levels.[72] Irrespective of algae growth method, whether photobioreactor or raceway pond, there will be significant energy costs for fans and spargers for the introduction of clean or flue gases to encourage rapid growth and aid carbon sequestration.[72]

1.2.10 Transesterification

Triglycerides are the major component of algae and vegetable oils. They can be transesterificated with alcohol over an acidic or basic catalyst to give a mixture of fatty acid alkyl esters (FAAEs) and glycerol. A stoichiometric reaction (Figure 1:5) requires 1 mole of triglyceride to 3 moles alcohol.[73] The temperature used for acid and alkali transesterification is reflux for the alcohol used.

Methanol is widely used as it allows the simultaneous separation, when water washings are used, of glycerol, catalysts, salts and soap; it is the cheapest of the alcohols and has low water content.[45] There can be problems with oil and alcohol miscibility when using acid catalysts however adequate stirring overcomes mass transfer issues.[74] The optimal ratio of alcohol to triglyceride, *i.e.* giving the highest ester yield, was twice that suggested by the stoichiometry (therefore 6:1 alcohol: triglyceride).[75] The water phase, during biodiesel

separation, is denser and separation can therefore be carried out at atmospheric pressure and room temperature.[45]

For completely sustainable production of biodiesel the use of methanol is not currently economically viable. However, methanol is preferable due to the capacity to process wet samples by the addition of more methanol to the transesterification reaction; a notable energy saving will be evident from the reduction in the energy intensive drying process.[76] Currently the most economic method to produce methanol is from syngas, which as an extract from natural gas, is non-renewable.[75b] Ethanol can be produced easily from renewable sources via fermentation thus producing a wholly green biodiesel production cycle.[75b] Unused, excess methanol can be re-processed using a one-step extraction and transesterification methodology for algal biodiesel production.[77] This reuse of methanol would further reduce the environmental and financial cost of algae biodiesel production.[77]

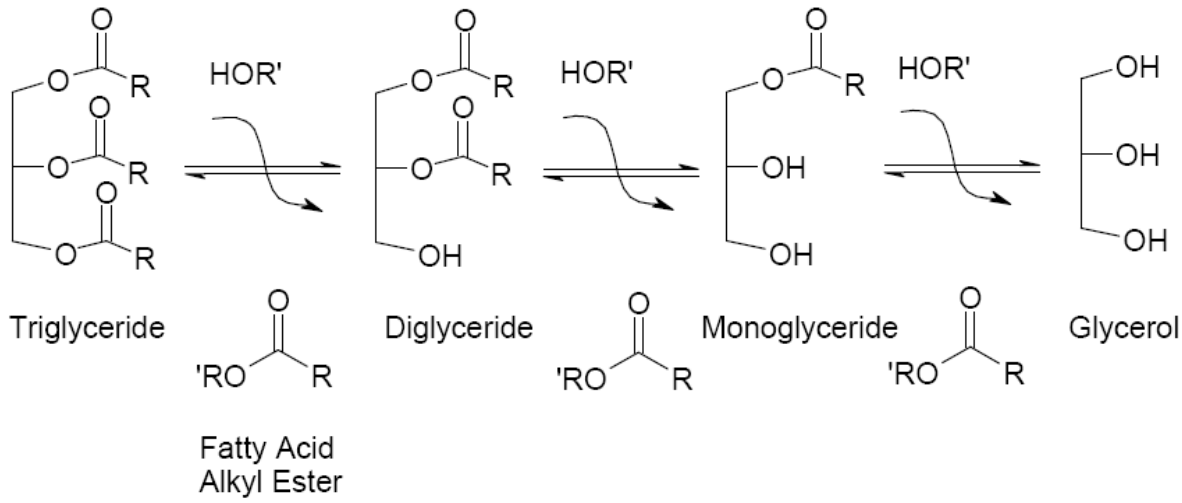


Figure 1:5 – Transesterification reaction

The proposed basic catalysis process is preferred in industry due to quicker reaction times and since the catalysts used (*e.g.* CH_3ONa) are less corrosive than acidic catalysts.[73] Saponification can occur with basic catalysis, particularly if water (>0.06 wt%) or non-esterified free fatty acids (>0.5 wt%) are present.[78] Despite this many companies still use and develop base catalysts, for terrestrial derived lipids, due to high conversions (>98 %) and cost effectiveness.[48] Unless algae are completely dry or has a low acid value, the use of base catalysts for production of algae will be unsuccessful.

Transesterification catalysed by Brønsted acids, *e.g.* sulfuric acid, give very high yields but reactions are slow (at least 3 hours).[73] Acid transesterification requires reflux temperatures

(around 70 ^{o}C depending on alcohol used).[73] The reaction for acid catalysed transesterification occurs to all lipids, not just the triglyceride.

The use of homogeneous catalysts require washing with large amounts of water to remove the catalyst used and the salt produced from the neutralisation process and thus batch processing is required.[48] Therefore the development of other heterogeneous catalysts, particularly metal catalysts, is desirable and has been reported in literature.[48] Heterogeneous catalysts often require higher temperatures and pressures than their homogenous counterparts. The harsher conditions used for metal catalysts are justifiable if they can be used continuously with no separation required from the biodiesel.[48] The increases in cost are offset by the reduction in up-stream processing of the biodiesel.[48] Zinc nanoparticles and lanthanum species have been shown to be particularly active for transesterification.[78b]

The successful use of metal amino acids, especially the use of zinc and aluminium, for transesterification has been proven with vegetable oils previously within the Davidson group as well as elsewhere.[19, 79] The stability of this kind of catalyst is important due to the potential reuse of heterogeneous catalysts and the use of metal amino acids adhered to monolithic supports. [79] These transesterifications run preferentially in the presence of water or free fatty acid and as such are ideal for algae derived lipid.[19] The active species within the reaction are unknown, but have been hypothesised to be metal glycerolates, carboxylates or methoxylates. The amount of catalyst leaching into the biodiesel layer is important to the application of such catalysts on an industrial scale.[79-80]

1.3 Microalgae

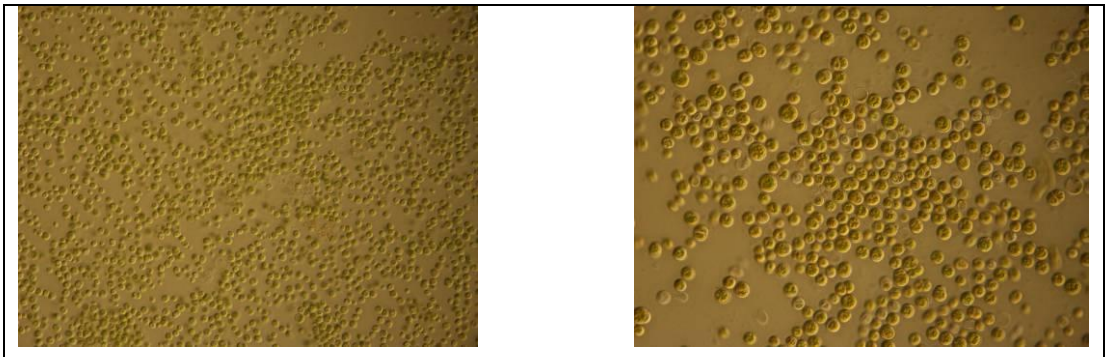
1.3.1 Microalgal species

There are more than 100,000 known species of diatoms, 40,000 known species of green plant-like algae as well as other types of algal species.[2] Different species have different lipid contents and biomass productivity; both are required to be high to produce biodiesel at a maximum rate.[81] Although diatoms are known to have a high lipid concentration they have a four membrane barrier between the stroma and the cytoplasm. This barrier makes lipid extraction an energy intensive and laborious process.[82] Species such as *Botryococcus braunii* produces up to 70 % of their weight as isoprenoids which are less oxygenated than

lipids.[54] These compounds can be used in existing oil refineries; however *Botryococcus braunii* is notoriously difficult to culture and extremely slow growing.[54] Other microalgae than *Botrycoccus*, such as *Dunaliella* spp. and *Chlorella* spp. can be expected to double in cell count over a 24 hour period.[50] Araujo *et al.* reported that the *Chlorella vulgaris* and *Chlorella gracilis* are the most suitable algae for large scale lipid production due mainly to their tolerance of highly saline conditions and high carbon dioxide.[40, 83] Species such as *Dunaliella* spp. and *Nannochloropsis* spp. grow favourably in marine conditions (see section 1.3.9). The *Chlorella* spp. grow with particular vigour, often dominating in outdoor freshwater situations, their hardy character makes them suitable candidates for mass cultivation for biodiesel production.[58]

Table 1:3 – Lipid accumulation in algal species

Algal species	**Average lipid accumulation / % dry weight**	
	Nitrogen sufficient	**Nitrogen starved**
Chlorella vulgaris[86]	25	42
Chlorella emersonii[86]	29	63
Botrycoccus braunii[87]	39	19
Dunaliella salina[86]	19	10



A B

Figure 1:6 – Algal species at x 40 magnification: (a) *Chlorella vulgaris* and (b) *Chlorella emersonii* (H. Smith-Baedorf)

Chlorella vulgaris is a robust, freshwater, unicellular, green algae which is spherical and has a diameter of between 2 and 10 μm. There are no flagella found on this species of algae.[84] *Chlorella vulgaris* has been studied for many years and has reasonably high lipid content (14-22 %); its growth can be well controlled.[85] It has a high specific

growth rate of around 0.25 h^{-1} under optimal conditions and is capable of growth in high carbon dioxide levels.[36b]

Chlorella emersonii produces higher biodiesel conversions than *Chlorella vulgaris* (Table 1:3), particularly when it is grown in slightly acidic conditions (pH 6.5) or low nitrogen conditions.[85, 88] Microscope pictures (Figure 1:6) highlight the difference in size between the physiologically similar species *Chlorella vulgaris* and *Chlorella emersonii*.

1.3.2 Nutrients

Algae require light, water supply, carbon as well as particular macro- and micro-nutrients for growth. The specific nutrients can be provided for *Chlorella* strains by making up Bold's Basal solution (Table 1:4 and section 4.10).[89] Under stress conditions algae form storage product such as lipid in preference to carbohydrates or proteins and growth is slowed. [90]

It is reported[33] that after carbon, nitrogen is the most important element for algae growth since it is involved in the primary metabolism of microalgae and a constituent of nucleic acids and proteins. The nitrogen concentration affects the lipid content of the algae; limiting the nitrate levels causes the accumulation of lipid at the expense of growth rate.[33] The source of nitrogen affects algae growth, for example, fast growing species have been shown to prefer ammonium to nitrate.[33, 54, 91]

Table 1:4 – Bold's Basal growth medium constituents[89]

Major constituents	Minor constituents
$NaNO_3$	$ZnSO_4.7H_2O$
$MgSO_4.7H_2O$	$MnCl_2.4H_2O$
NaCl	MoO_3
K_2HPO_4	$CuSO_4.5H_2O$
KH_2PO_4	$Co(NO_3)_2.6H_2O$
$CaCl_2.2H_2O$	H_3BO_3
	EDTA
	KOH
	$FeSO_4.7H_2O$
	H_2SO_4 (conc)

The third most important nutrient for algal growth is phosphorus which is not always bio-available, often due to combination with metal ions, and thus required in excess.[33] Trace metals, particularly zinc, copper, manganese, molybdenum, boron and iron, are also effective for algae cultivation.[33]

1.3.3 Growth parameters

The growth of algae is measured by the specific growth rate (h^{-1}); the fraction increase in biomass over unit time.[47a] To begin with the growth has a lag time as the specific growth rate is below the maximum, however once the algal cells have adjusted to the conditions there is exponential growth (Figure 1:7). After the exponential growth has occurred there will be linear growth as the quantity of light energy absorbed is determined by cell concentration – not photon flux density.[47a] Each algal species has a characteristic photosynthetic rate and thus specific growth curve for lag, exponential, stationary and death growth stages which are all affected uniquely by an increase in light intensity with increase temperature.[22a, 92]

1.3.4 Growth rates

All crops have varying growth rates depending on their growth conditions and processing.[93] Over sixty years of research suggests that it will not be possible to produce more than 100 tons ha^{-1} yr^{-1} at a commercial scale with current technology and the strains of algae available.[29b] Many different aspects of cultivation affect the growth rate of algae, as well as each species having differing optimised conditions for high growth rate. Algae have a high growth rate and year-round growth; it can produce 10 to 100 times as much biomass as terrestrial plants in a year.[22a, 29b, c, 50]

The growth rate for algae on pilot and larger scale is generally measured as grammes per litre medium accumulated over a set period of time.[47a] Growth of algae is often measured by mass or the average cell count. However caution must be exercised since the mass may appear to reduce immediately after mitosis of the mother algae due to the daughter cells being smaller and thus lighter than the mother cell. This will give a slightly lower cell distribution as the average cell mass decreases when mitosis occurs. The average cell mass increases once sufficient time and conditions have encouraged the mitotic state to be reached for the daughter cells.[94]

Combining the effects of photosynthesis, irradiance distribution, temperature, fluid dynamics, and carbon and other key nutrient bioavailability, growth rate is particularly sensitive to changes in environment. Optimisation of growth rate yields varying results for the many species of algae. Often 5 – 20 wt% lipid is observed for algae growing in optimised conditions, whereas under unfavourable proliferation conditions the lipid content often increases to 20 – 50 wt%.[22c] It has, therefore, been hypothesised that algae should be initially grown in optimised conditions and later exposed to stress conditions to increase lipid yield for the production of biodiesel.[42]

The cell density in open ponds reaches a maximum 0.5 – 1.0 g L^{-1} of dry cell weight even under optimised (carbon dioxide enrichment), which means that immense land and water resources would be required for algal biodiesel to replace petroleum diesel.[2] Chisti *et al.* reported theoretical algae oil yield of 1.55 – 3.62 gallons per acre per year.[22a, 66]

1.3.5 Algae growth conditions

Algae are commonly grown phototrophically (photosynthetic growth) due to their efficient photosynthetic energy conversion of even limited light sources; however it is possible that their uptake of carbon and subsequent growth rates can be increased by mixotrophic growth.[40, 95] Most algae are able to grow mixotrophically, *i.e.* in the presence of a light source and a fixed organic carbon source, such as acetate or glycerol.[96] See section 1.3.2 for more information of algae nutrient requirements and section 1.3.8 for more about mixotrophic algae growth.

Mixotrophic growth (section 1.3.8) has been reported to increase biomass productivity in algae species, particularly when the additional carbon source was introduced in a secondary stage of growth. An increase of 72 % for FAME by volume of growth medium was seen by Das *et al.* for *Nannochloropsis* sp. when cultivated with 2 g L^{-1} glycerol in phototrophic conditions, compared to solely phototrophic growth.[96] The carbon source which creates the environment for highest intracellular lipid levels in mixotrophic conditions is specific for different species of algae. For example for *Chlorella* spp. glucose is appropriate, whereas for *Phaeodactylum tricornutum* glycerol is preferable, as well as for *Nannochloropis* spp., as mentioned earlier.[96] These mixotrophic cultures have all been cultivated in sterile closed laboratory systems, however in open systems, with the additional carbon sources it is expected that heterotrophic bacteria may present a

contamination issue and impact on the algae yield.[96-97] There is also precedence that some algal species co-exist with fungi and bacteria particularly favourably.[98]

Algal biofuel production consists of upstream and downstream processing. The upstream processing or feedstock production includes algae growth, carbon dioxide sequestration and lipid extraction; incorporating algae effluent collection and concentration to slurry and then to cake prior to lipid extraction.[42, 99] The downstream processing or biodiesel production consequently covers the lipid pre-treatment and subsequent transesterification alongside separation and preparation of biodiesel for end use.[42, 99]

1.3.6 Effect of carbon sources on algal growth

Organic and inorganic carbon can often be a costly solution to increase lipid content and growth rate. Algae usually process agricultural waste ineffectively possibly due to it containing more complex compounds such as proteins, polysaccharides and long chain fatty acids. Algae have been reported to utilise the simpler molecules of glycerol and acetate by accumulation of increased lipid content and overall biomass.[97a] Some research has shown that mixotrophic growth can significantly increase lipid content within algae biomass compared to phototrophic growth alone. For example Xu *et al.*[100] who reported *Chlorella protothecoides* 15 % lipid for phototrophic growth compared to 55.2 % lipid content with the addition of an organic carbon source, corn powder hydrolysate.

Heredia-Arroyo *et al.*[15] analysed the effect of heterotrophic (growth using exclusively organic substances), phototrophic and mixotrophic growth conditions on *Chlorella vulgaris* in batch cultures. The highest growth rates were observed at 48 hours for heterotrophic (1.88 increase in biomass concentration *cf.* phototrophic) and mixotrophic (3.5 increase) growth conditions with an initial glucose concentration of 4 g L^{-1}.[15]

1.3.7 Algal growth: the interrelated effects of carbon dioxide and pH

Temperature, light, pH, NO_x and SO_x species of algae, density of culture and concentration of carbon dioxide all affect the carbon dioxide sequestration process for algae.[98b] Different growth parameters are interrelated in their effect on growth rate and lipid accumulation, however an increase in carbon dioxide levels generally increases growth rate up to a certain point.[101] The algae have a species specific tolerance limit of carbon dioxide as excess can be detrimental to growth. The carbon dioxide negative impact is due to

decreased pH levels which are impacted by the chemical form of carbon. At high carbon dioxide concentrations there is a biological reduction or inhibition in algae cells capacity for carbon dioxide uptake. Once at high carbon dioxide levels, algae previously exposed to lower carbon dioxide concentrations display an increase in cell growth.[102] For *Nannochloropsis oculata* an increase of sparged carbon dioxide from 0.03 % - 2 % increased the cell growth by almost three times and maximum high cell concentration is increased by 4.75 times.[103] However further increase in the carbon dioxide fraction to 5 % completely inhibited algal cell growth; attributed to the associated lowered pH.[92, 104] For different species high carbon dioxide concentrations are plausible, for example, *Botryococcus braunii*.[105] *Chlorella vulgaris* treated with 4 % carbon dioxide displayed large variability in pH levels.[106] The highest levels of carbon dioxide were associated with increased growth rate and biomass concentrations.[106]

In aqueous systems, inorganic carbon is available in a variety of forms including, carbon dioxide$_{(aq)}$, H_2CO_3, HCO_3^- and CO_3^{2-}, which are all interconvertible via dynamic exchanging reactions dependent on temperature and pH.[107] Outside of a particular threshold pH becomes difficult to control and chemical precipitation of salts that damage the algae and cause deterioration of the medium occurs; due to the formation of CO_3^{2-}, OH^- and PO_4^{3-}.[29c] pH limitation is more detrimental to the algae than light limitation as it affects the biochemistry of the growing media. pH limitation affects the ionic absorption from the growing media and the metabolic biochemistry of the algae cells.[29c] Carbon dioxide is used as the carbon source for phototrophic algae as it is easy to control in closed systems, allows photosynthetic growth and causes only minor pH changes.[108]

Algae species have different optimal pH's, with most preferring neutral pH, though those such as *Spirulina platensis* prefer higher pH and others prefer slightly acidic pH, including *Chlorella vulgaris* and *Chlorella emersonii*.[109] During algae growth pH can change in the growth medium, the addition and subsequent chemical equilibrium of the carbon dioxide decreases the pH of the medium whilst algae biomass growth increases pH.[109] The algal growth usually has higher rates of influence on pH than acidification by introduction of carbon dioxide on the growth medium.[109] pH also affects other nutrients such as the balance of NH_3 and NH_4^+ and influence on oxidation reactions leading to oxygen production.[33]

1.3.8 Mixotrophic algae growth

Algae can grow either with light - phototrophically, in the dark – heterotrophically, or mixotrophically. Mixotrophic growth is the culture of algae using both assimilation of organic compounds as carbon sources and inorganic compounds as electron donors; *i.e.* growth using both photosynthesis and more complex carbon sources. The metabolism of carbon from different sources is possible from varied sources such as dissolved inorganic carbonates, sugars, molasses and acetic acid. Atmospheric carbon dioxide levels do not allow optimal growth of algae and as such a supplementary supply is required.[33] The use of heterotrophic growth removes the loss of growth due to shading at higher cell concentrations leading to a potential to increase biomass production and productivity.[110]

It is observed in literature that some algae species show superior growth when the synergistic effects of both carbon dioxide fixation and carbon substrate addition are present.[110b] *Chlorella protothecoides* has been shown to yield 69 % more lipid with the addition of glucose.[110b] The supplementation with glucose does increase the cost of algae fuel production therefore cheaper carbon sources are of interest, for example, glycerol which is a by-product of the biodiesel process.[110b]

In literature, Das *et al.*[96] cultivated *Nannochloropsis* sp. using atmospheric carbon dioxide only for the first eight days. On the ninth day 1 g L^{-1} glucose, sucrose and glycerol were each added to the different samples, one sample was reserved as a control.[96] The addition of glycerol gave highest overall yield of FAME, after 12 days of culture, with the control, glucose and sucrose all giving similar percentages of FAME as a percentage of dry weight.[96] The net biomass productivities did vary compared to the control, with mixotrophic growth yielding 87, 103 and 100 mg L^{-1} for glucose, sucrose and glycerol respectively, whilst the control sample gave just 27 mg L^{-1}.[96]

The concentration of alternative carbon source affects the rate of uptake, for example, for *Chlorella vulgaris* increased glucose concentration yielded higher biomass and if the growth medium was not saturated with glucose, the glucose present was consumed more rapidly.[15] There were significant differences found in biomass concentration accumulated though no difference for the specific growth rates or in the percentage lipid at varying glucose concentrations.[15]

Glycerol is a by-product of the biodiesel reaction and therefore would be a convenient choice for carbon source for algae growth. Growth inhibition for *Chlorella vulgaris* was observed in literature at glycerol levels above 20 %.[15] However using a mixed carbon source of 80: 20 % glucose: glycerol increased algal biomass concentration. The lipid weight percentage of the algae increased with higher levels of glycerol; however this was offset by the reduction in overall productivity.[15]

Sodium bicarbonate has been reported to increase growth rates of *Chlorella vulgaris* due to its high solubility increasing retention time in growth solution, yielding 0.55 g biomass per gramme $NaHCO_3$.[111]

Mixotrophic conditions make available the sequestration of carbon from both organic and inorganic sources whilst maximising biomass and lipid productivity.[15] This use of alternative carbon sources is also useful for wastewater treatment where effluent can be turbid and non-permeable by sunlight; mixotrophic growth could make water treatment energetically and environmentally feasible.[15]

1.3.9 Salinity effects on algae growth

Nannochloropsis spp. have been reported to not be significantly adversely affected by increased salinity in the growth media; therefore evaporation and high salt levels should not be a major issue for *Nannochloropsis* spp. growth.[96] For species such as *Chaetoceros gracilis* the higher salinity environments cause a reduction in yield and thus lowered oil productivity to less than four times.[40] Whilst for other species, such as *Chlorella vulgaris* increasing salinity from 25 g L^{-1} to 35 g L^{-1} (seawater salinity levels) increases the yield of biomass from 0.0415 g L^{-1} growth media to 0.2624 g L^{-1}, thus increasing the oil production by 6.4 times.[40] The increase of sodium chloride concentration does, however, inhibit *Chlorella vulgaris* growth in mixotrophic cultures.[15] Cultivation of halo-tolerant algae in saline conditions could minimise the growth of invasive bacteria and algae.[96]

1.3.10 Effect of limiting conditions on lipid content of algae

To enhance lipid and thus biodiesel production, the biomass productivity (production per unit volume per unit time), cellular lipid content and overall lipid productivity need to be increased. The effect of different environmental conditions has been observed for algae species in the rate of biomass growth, percentage lipid and fatty acid profile of the resultant

biodiesel.[83, 112] Parameters which can put the algae under stress include nitrogen[2, 29b, 90b, 98b, 113] phosphorus[21b, 29b, 114], silicon[86], salinity[115], and iron[116]. The different growth conditions are not always compatible and so optimisation is required. Under limiting conditions algae have been shown to produce increased lipid accumulating in the cytoplasm in preference to cell division and growth due to a shift in biosynthetic pathways.[88] The inhibition of cell division can lead to lower overall lipid productivity, despite the high lipid percentage per cell, due to the lower biomass productivity during nutrient starvation.[2, 87, 106-108]

The increase in lipid content in algae can be achieved by limiting the nutrient availability, extremes of temperature and pH or by the use of irradiance.[22c] These stresses in different algae species cause extremely varied results, for example *Chlorella* spp. accumulate lipid under nitrogen starvation whilst *Dunaliella* spp. lipid content diminishes under the same conditions.[117]

Initial algae literature research highlighted phosphate and nitrate levels as important parameters for algae growth due to their widely varying levels in water and the implications for organism growth.[93a] Increased levels of phosphates and nitrates which are known to be required for cellular growth are present in water from recently fertilised fields and municipal wastewater. Nitrate level is of particular interest for algae grown for biodiesel since studies in literature have highlighted that reducing the amount of nitrate available to the algae causes stress conditions which encourage the higher accumulation of cellular lipid.[90b] Nitrogen constitutes largely to the essential, intrinsic cellular matter of algae, such as in protein, pigmentation, nucleic acid and cell wall elemental make-up.[118]

Under nitrogen starved phototrophic conditions algae can fix carbon via photosynthesis. However the absence of nitrogen leads to the algae being unable to metabolise the fixed carbon for protein synthesis resulting in a metabolic shift to intracellular production of lipids.[96, 119] Scragg *et al.*[98b] reported that *Chlorella vulgaris* and *Chlorella emersonii* both yielded an increased amount of lipid under nitrogen limited conditions compared to nitrogen sufficient conditions, 28 % to 58 % and 25 % to 34 % increase respectively. The specific growth rate of *Chlorella vulgaris* diminished from 0.69 day^{-1} to 0.12 day^{-1} and a change in lipid content of 28 – 58 %. For *Chlorella emersonii* the growth rate was unchanged regardless of nitrogen levels.[98b] In some cases effects of nitrogen starvation can lead to a reduction in growth rate of the algae which is not offset by the higher lipid

fractions obtained and therefore the stress conditions do not necessarily translate into higher lipid productivity.[120] The source of nitrogen also has an impact on the amount of lipid produced in algal cells.[113a]

When *Nannochloropsis* sp. was grown under normal and nitrogen starved conditions with high light and low light conditions it was reported that nitrogen starvation reduced the overall biomass by half.[121] The high light conditions enhanced growth regardless of amount of nitrogen available and the highest average lipid productivity was obtained with high light, reduced nitrogen and decreased salinity due to reduced cell division in saline-stressed cells.[103, 121] The accumulation of lipid within the nitrogen starved algae cells is observed due to the continuous carbon supply exceeding the photosynthetic capacity of the stress-limited cells.[103]

Converti *et al.*[122] reported that varying the amount of nitrates for *Chlorella vulgaris* and *Nannochloropsis oculata* had no significant effect on the amount of C16(0) present in the resultant fatty acid methyl esters (FAMEs) produced. It was noted that reducing nitrate concentration led to an increase in the C18(3) concentration for *Chlorella vulgaris* samples. A 75 % reduction in nitrate concentration did not affect the growth rate of the algae, though it produced a doubling of overall lipid content.[122]

Phosphate is required for building molecules integral to the photosynthesis reaction and as such is a key macroelement for algal healthy growth and development. It is required for many of the metabolic processes of algae and is present in ATP, DNA, RNA and cell membranes.[118] Inorganic phosphate as $H_2PO_4^-$ and HPO_4^{2-} are accredited in literature with being the most easily processed phosphate forms.[118] Bold's Basal media contains these inorganic phosphates in a dynamic equilibrium in the form of potassium salts. Inorganic phosphate allows for pH buffering due to their dynamic equilibrium.[118] It is imperative to supply phosphate in significant excess as certain phosphate compounds are not bio-available.[33]

Iron has been reported to, at high concentration, induce lipid accumulation particularly in *Chlorella vulgaris*.[110a]

Temperature regulates cellular, physiological and morphological changes in algae. An increase in temperature can accelerate metabolic rates in the algae whilst lowered temperatures inhibit growth.[33] Optimal temperature varies with species, and even species grown at elevated temperatures in their natural habitats, such as at the Roman Baths - Bath UK, may have lower optimal growth temperatures. Generally, green algae species are best cultivated in the range 25 – 35 ^{o}C, and for *Chlorella vulgaris* it has been reported that there is maximal lipid production at 25 ^{o}C.[34] However it has also been reported that temperature had little effect on the FAME profile obtained for *Chlorella vulgaris* though a reduction from 30 ^{o}C to 25 ^{o}C gave a lipid content increase of 250 %.[122] It has been reported that the dark temperature of phototrophic algae does not dramatically affect the growth rate since photosynthesis cannot occur in the dark; optimal temperature is, therefore, influenced by light intensity.[33] Generally algae grown at higher temperatures yield a higher percentage of monounsaturated and saturated FAMEs compared to those grown at lower temperatures.[117a] This is exacerbated in cultures exposed to long periods of increased light intensity.

Lipid accumulation is also affected by the growth phase of the algae.[103, 123] It has been observed that after optimum growth stages, algae left without harvesting began to excrete oils and lipids into the growth medium. It has been reported that total biomass concentration can still increase after cell division has ended due to carbon metabolisation which results in the diversion of carbon to lipid production.[124] The various stages of algae growth: (i) lag stage - where the culture acclimatises to its new growth conditions; (ii) logarithmic – where exponential algae growth occurs due to rapid cell division in ideal growth conditions and plentiful nutrients, carbon sources and light; (iii) early stationary – where the algae culture reaches growth medium saturation due to nutrient or carbon source deficiency, or the light penetration through the culture is insufficient; and (iv) late stationary phases – where the deficiencies in the previous stage, iii, limit growth to the point of cellular death (overviewed in Figure 1:7). It has been reported that the growth phase of the algae has a much larger effect on biochemical composition than nitrogen source.[125]

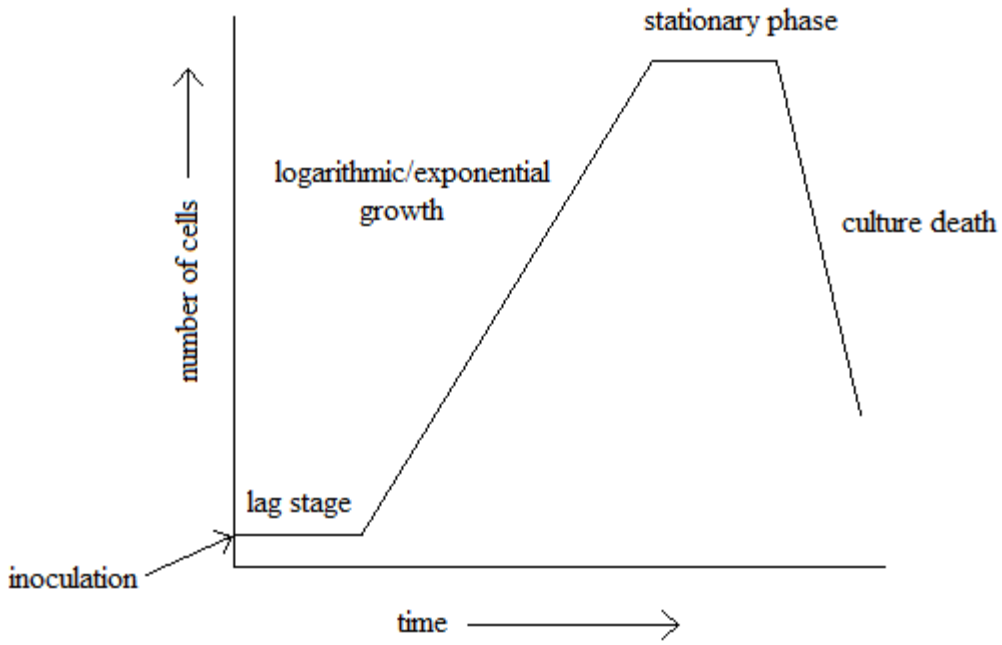


Figure 1:7 – Simplified overview of algal growth

Other potential areas to increase the lipid productivity of the algae would be to increase the photosynthetic efficiency of the algae.[126] This could be achieved by metabolic engineering, though this is not focussed on here. Other routes are the maximising yield and productivity by creating a continuous process. Another way to increase lipid production is through the design and successful use of an economic, efficient photobioreactor which minimises solar reflection whilst optimising photon capture by the algae cells, increasing mass transfer and thermal control.[126]

1.3.11 α-tocopherol and carotene

The presence of vitamin E in chloroplasts improves the longevity of biodiesel produced from algae.[102b] This hydrophobic antioxidant vitamin E (also known as α-tocopherol) is usually maintained in a reduced form by an associated ascorbate which allows the lipid soluble α-tocopherol to be extracted during lipid extraction for biodiesel formation.[102b] The presence of α-tocopherol in biodiesel produced from algae allows it to continue to influence oxidation.[56, 102b] Within the chloroplasts of the algae, α-tocopherol in high concentration, prevents lipid peroxidation as it removes singlet oxygen and lipid peroxyl radicals; it appears to act as an anti-oxidant in biodiesel too.[102b]

It is hypothesised that there is a residual pool of reactive oxidative species in algae as well as higher plants and that this pool increases when the plants undergo stress, potentially for signalling purposes (see section 1.5.12).[104a]

Carotene is produced by algae which are exposed to high light environments as a photo-protective molecule. The increase in carotene content in an algae culture is a good indicator that there is adequate recovery of the culture to high light.[104b] Contrastingly, in low light situations, algae acclimatise by producing increased chlorophyll α molecules to harvest the maximum amount of light. The chlorophyll α: carotene ratio can be useful to determine whether an algae culture has acclimatised to a specific photo-situation.[104b]

1.4 Downstream processing and extraction of lipids from algae

1.4.1 Separation of algae from growth media

Algae require separation from the growth medium to be usable for biofuel and other products. This usually involves one or more solid-liquid phase separation steps such as gravity sedimentation, flotation, centrifugation, screening, filtration, electrophoresis or flocculation.[127] The difficulty in separation of algae from the culture medium is mainly due to their small size, 3 – 30 μm, and their relatively low concentration in the growth medium, often below 500 mgL^{-1} in industrial processes.[127] Another reason for the algae cells suspension throughout the growth medium is due their electronegative surface charges (at pH 2.5 – 11.5) and low specific gravity.[64e] To increase the concentration of algae cells within the photobioreactor is important to ease harvesting and increase efficiency of biofuel production.

Processing of algal matter is one of the major stumbling blocks to its biofuel commercialisation along with cultivation and harvesting. The drying step is a bottleneck in the algal biodiesel process and uses energy in vast amounts due to the high latent heat of vaporisation of water.[110a] Since lipids are immiscible in water they do not require separating from aqueous solution. However since lipids are accumulated intracellularly in algae which mixes well with water this causes extraction issues prior to processing.[128] Different process systems use varying cultivation techniques due to the process being optimised for particular species, photobioreactor and the different uses of the algae.[99, 129]

Centrifugation is the use of enhanced gravitational force to increase the rate of sedimentation. The success of centrifugation and the conditions required depend on the biomass residence time within the centrifuge field, the biomass settling rate and settling distance.[130] Choice of centrifuge shape is determined by the amount of algae to be harvested.[127, 130] Centrifugation with a preceding flocculation step to improve recovery has been suggested to be the best way to process most algal products to reduce process energy.[131]

If the algae being recovered are particularly fragile and small, centrifugation can shear cell walls and flocculation can chemically contaminate the biomass, thus microfiltration or ultrafiltration can be used. Ultrafiltration can be used for the collection of product metabolites.[128] Any kind of filtration requires regular cleaning due to blocking so this is not an ideal method. For larger microalgae rotary vacuum drum filtration and chamber filter press are often used.[127]

Wet slurries of algae which are left over a few hours can spoil and degrade, thus adequate drying methods are required. Complete drying of the algae commercially is usually achieved by spray drying prior to recovery of metabolites or other desired compounds is costly, thus it is preferable to use a moist biomass paste.[128] On a laboratory scale different techniques are used for dewatering, one of the most rigorous is freeze-drying.

Lyophilisation (or freeze-drying) is the most gentle of all drying methods and allows the ice crystals from centrifuged slurry to be sublimed by slight warming without thawing.[132] This is carried out by exposure to a partial pressure of water vapour below 4.6 mmHg, the triple point of water. Below this pressure the addition of heat converts ice directly to water vapour. The sublimation of the ice leaves cavities within the algae where reabsorption of water is rapid. This technique is energy intensive but sensitive to the algae cells and very effective.[127]

Biomass concentration is most often analysed by dry weight measurements. An alternative technique is spectrophotometric method has been developed to allow fast and accurate measurement of biomass concentration.[133] Filtering of the sample and re-dissolving in a known amount of distilled water allows for accurate determination of cell concentration. A calibration curve can be plotted and for lower optical path penetration there is a higher

count.[133-134] Typical biomass concentration in open ponds lies between 0.4 – 1.0 g dw L^{-1} and up to 4.0 g dw L^{-1}.[22a, 135] The composition of the biomass is influenced by dilution rate, the higher dilution rate of the algal cells in the growth medium the higher the protein and carbohydrate content of the biomass and thus the lower the lipid content.[135] As the dilution rate decreases, due to algae growth, energy is mainly directed towards the growth and storage of energy as lipids.[27]

1.4.2 Lipid extraction utilising solvents

The majority of lipids extracted from algae cells are triglycerides. The remainder of the lipids include phospholipids, sterols, stannols, steroids diglycerides, monoglycerides, sulfolipids, glycolipids, carotenoids, hydrophobic compounds and vitamins.[2, 27, 136] The reduction in lipid content is largely due to the decrease in neutral lipids formed, structural lipid content (galactolipids and phospholipids) stays more constant.[133] Neutral lipids are mainly saturated and monounsaturated fatty acids can decrease by 45 %, and polyunsaturated fatty acids, which constitute more structural lipids, decrease by 34 %.[133]

Traditional lipid extraction methods include pressing where no solvent is used; the disadvantage is that a large, dry sample of algae is pressed over hours.[137] Extracting compounds desired by degradation of the cell walls can be carried out using a variety of more modern methods including: (i) solvent extraction (using hexane, methanol, chloroform or supercritical carbon dioxide); (ii) ultrasonics; and (iii) salt imbalance.[90a]

Solvent extraction is a popular method which requires relatively inexpensive solvent producing reproducible results, regardless of solvent used there are issues with solvent recovery and flammability. Soxhlet extraction is a popular method in literature and uses solvent evaporation, subsequent condensation and percolation cycles to extract the lipid less intrusively from the solid algae biomass; this technique works well for small samples.[21a] Soxhlet extractors are used when the desired compound has a limited solubility in the solvent and the impurities are insoluble in that particular solvent; thus no further separation techniques are required.[138] If the desired compound has a high solubility in the solvent then regular filtration can be used.[2]

Ultrasonic assisted solvent extraction is effective for the extraction of lipids from tougher algae cell walls which reduces extraction time.[137] Precautions must be taken with

ultrasonic assisted solvent extraction since it is a highly energy intensive process and has scale-up issues. Supercritical fluid extraction is a non-toxic method however currently it has prohibitively high energy and economic costs.[137] Other extraction methods are feasible and available.[139]

In literature the most effective solvent for extracting the highest percentage of lipid from algae cells has been reported as chloroform and methanol at a ratio of 2:1 by volume, this is followed by methanol, chloroform, ethanol, and then hexane.[14] The $CHCl_3$: MeOH (2:1 ratio) solvent is a positive azeotrope – the boiling point of the mixture is below both boiling points of the constituent solvents. Distillation will not affect the constitution of the azeotrope and neither does heating, so it cannot be separated in this way.[140] The composition and content of the lipid fraction extracted from the algae could be affected by the polarity of the solvent and the extraction method.[14] The use of consecutive solvents with varying polarity has realised increased extraction rates. If non-polar solvents are added first they can weaken the association of lipid with the algae cellular structure and the subsequent addition of more polar solvents to draws out the lipid from the cell. With wet biomass it is suggested that water forms a solvent shell around the lipids lessening the efficacy of extracting non-polar solvents.[14, 141] To increase the extraction efficiency disruption of the cell wall and permeability are key.

Accelerated solvent extraction uses pressure to increase the rate of solvent extraction. It has been shown in literature to recover total lipid from algae and oleaginous yeast species which have easily permeable cell walls such as *Chlorella vulgaris* and *Rhodotorula glutinis*.[138, 142] It allows the use of up to 20 times less solvent and is up to 5 times faster than traditional solvent extraction methods. It is not, however, usable for all species of algae and hence is not used in literature for *Chlorella* species for lipid extraction.[138, 142] Care is required when drying lipids extracted using any method due to their potential decomposition at high temperatures.[117b]

1.4.3 Alternative extraction techniques

The development of an all in one extraction and transesterification step for algae would cause a notable reduction in the processing and energy costs. Direct or *in situ* transesterification of algae avoids potential lipid loss due to increased processing of the algae leading to higher yields of FAME. Ehimen *et al.*[143] successfully carried out *in situ*

transesterification using dried *Chlorella* algae biomass, sulfuric acid and methanol. Lewis *et al.*[144] used predominantly methanol with hydrochloric acid and chloroform to allow for transesterification and lipid/biodiesel solubility respectively of their thraustochytrid strains. The FAME profile did not vary significantly whether the two step method or all in one methodology was used. Another *Chlorella* spp., *Chlorella pyrenoidosa*, was extracted and transesterified in both a two-step and a one-step fashion, the two step process resulted in a higher FAME yield.[21a] Zimmerman and Soh increased lipid extraction by 20 % by using an initial bead beating or microwaving step to extract lipid from an un-named algal species[77a, 132] Patil *et al.*[76] employed an optimal ratio of 1: 12 algae: methanol with 2 wt % potassium hydroxide catalyst and domestic modified microwave for 4 minutes.[76] The all-in-one process is limited by excess sulfuric acid and alcohol, which would also increase solvent recovery costs. The use of methanol in the extraction stage of the two step process has been reported by D'Oca *et al.*[21a] to eradicate the solvent recovery step between extraction and transesterification compared with traditional hexane extraction.

Microwaves have also been used to extract triglycerides from algae using conditions such as 100 mg algae, 1.8 wt % sulfuric acid and 2 mL methanol at 80 °C for 20 minutes. Phase separation was achieved with the addition of chloroform and water.[77a] The success of microwaves for the transesterification of algal lipid has been reported too.[145]

The use of microwaves to produce biodiesel effectively has been reported in literature compared to the largely inefficient mechanical extraction methods which are hindered due to algae's rigid cell walls.[146] Reaction times can be significantly reduced and yields increased by using microwave technology compared to conventional reaction methods.[77b] Less solvent is required than for conventional methods and fewer by-products are obtained; lowering overall energy consumption. The constant changing of the electromagnetic field in polar molecules (such as water and alcohols, particularly methanol) due to microwave irradiation activating the smallest degree of variance is the key to microwave extraction and transesterification efficacy.[146] Varying the electrical field interacts with the molecular dipoles and charged ion which leads to rapid rotation and heating due to molecular friction. The success of microwaves for the lipid extraction is attributed to the accelerated heat and pressure formed inside of the algae cell which can force out the extractions. This can also

aid the degradation of the cellular wall components, such as algin and increase mass transfer rates for the transesterification of the triglycerides.

Microwave biodiesel production has been carried out using microwave irradiation using both heterogeneous[129, 147] and homogenous[148] catalysts. Barnard *et al.*[130] reported that the use of a continuous flow microwave for transesterification is more energy efficient than traditional transesterification methods. One of the major issues with microwave technology is the scalability from laboratory analytical scale to industrial scale mainly due to the penetration depth of microwave radiation.[149]

The FAME profile of the lipid extracted from the algae is affected by the extraction and transesterification processes it undergoes. *Scenedesmus obliquus* (a green algae) had lipid extracted using a purpose built continuous microwave system at 95 ^{o}C for 30 minutes.[150] Further processing of the lipid was required to synthesis FAMEs. Interestingly when the lipid has been transesterified it contains 72.92 % unsaturated FAME, whilst Soxhlet extracted lipid when converted to FAME has 85.95 % saturated FAMEs.[150]

On a larger scale open-vessel microwave (CEM discoverer) experimentations were carried out using soybean oil, 1 wt % potassium hydroxide and methanol. These were able to process up to 3 kg oil per batch, quantitatively converting it to biodiesel by heating the reaction mixture to 50 °C and holding for 1 minute.[151] Using the same microwave Wahlen *et al.*[77b] identified that fundamental parameters affecting reaction time and efficacy are the ratio of alcohol and triglyceride, the type of alcohol used, the temperature and the concentration of acid catalyst. The longer chain alcohols, such as n-propanol and n-butanol are more miscible with the triglyceride and give higher reactivity than methanol and ethanol.[77b] Higher reaction temperatures can also be used with the longer chain alcohols due to their higher boiling points; this increases efficiency and speed of transesterification whether using microwave or more conventional heating methods.[77b] The use of longer chain alcohol does cause a rise in the cost of solvents used and a change in the properties of the resultant fatty acid butyl esters (FABEs), compared to their respective fatty acid methyl esters (FAMEs). Initial studies suggest that the fuel properties of FABEs are comparable to those of the relevant FAMEs, improving qualities for cold weather applications.[77b]

Another method, bead milling can be used to rupture cell walls to release products; this is a crude method and chromatography is required to recover high value products at high purity.[90a] Extraction of lipids at lower temperatures (0 – 60 °C) is more successful than extraction at higher temperatures (80 – 100 °C).[139]

Lee *et al.*[152] compared five methods of lipid extraction upon three algae species to find the most effective method. The algae species were *Botryococcus* spp., *Chlorella vulgaris* and *Scenedesmus* spp. the lipid percentages extracted for each method varied as shown in Table 1:5.

Table 1:5 – Lipid extraction methods [152]

Extraction method	**Lipid contents / g L^{-1}**
Autoclave	5.4 – 11.9
Bead-beating	7.9 – 8.1
Microwave	10.0 – 28.6
Sonication	6.1 – 8.8
10 % NaCl solution	6.8 10.9

It is concluded from this study that microwave lipid extraction is the simplest and most efficient method for use with algae samples, potentially due to the sufficient disruption of cells during this process.[152]

1.4.4 Fatty acid methyl ester profile analysis

Gas chromatography mass spectrometry (GCMS) fatty acid analysis is an essential analytical method for quantitative, high resolution, reproducible results – particularly with dry algae samples of < 0.5 g.[153] Wahlen *et al.*[77a] transesterified algal biodiesel *in situ* using 2 mL methanol, 2.0 wt% sulfuric acid and 100 mg algal biomass at 80 ^{o}C in a microwave for 20 minutes for GCMS analysis. The *National Institute of Standards and Technology* 2005 mass spectral library was used to identify the peaks.[77a] *Dunaliella tertiolecta* pyrolysed bio-oil was also analysed by GCMS where the analysis of hundreds of peaks was attempted, some of the peaks were not separated or well defined; highlighting the complexity and challenges of algal bio-oil.[154] For bio-oil formed from *Nannochloropsis* sp. the GCMS results gave the five most abundant compounds as palmitoleic acid, palmitic acid, heptadecane, neophyadiene and cholesterol. This GCMS analysis was thought to be incomplete due to poor elution of higher molecular weight compounds and thus

transesterification of bio-oil into biodiesel or the use of (high pressure liquid chromatography mass spectrometry) HPLCMS analysis may be more suitable.[155]

1.5 Photobioreactors

Successful microalgal reactor design requires consideration of contamination, mixing, gaseous exchange, nutrient supply, light source (quality and quantity), pH and temperature control, building material, inlet and outlet for biomass and geometrical configuration.[108] There have been many and varied reactor designs for microalgae which have been constructed and used with differing success. This review will cover the main parameters determining algal growth and lipid accumulation within photobioreactors.

1.5.1 Considerations for photobioreactor design

One of the most important initial considerations for an algae photobioreactor is the source of light to be utilised, this will depend on the location of the photobioreactor particularly if non-arable land is used. In Europe those within the 45 – 30 ^{o} N window are most suitable for natural light illumination whereas elsewhere supplementary illumination and heating will be required.[56, 156] The maximisation of light exposed area is important, regardless of the light-source, for increased growth rates.[56] Many photobioreactors are vertical to increase the illuminated area of algae for unit land mass. Cultivation of algae has been achieved in open ponds as well as horizontal and vertical tubular reactors, flat plate reactors, membrane reactors and airlift reactors.

These photobioreactors incorporate differing designs, such as helical-coil designs and flat panels to optimise between natural ventilation and light utilisation.[157] A combination of natural and artificial illumination in helical-coil reactors was found, for example, to be optimal for *Nannochloropsis* growth. The *Nannochloropsis* sp. was, in this case, found to tolerate increased temperatures of up 40 °C. The construction materials of the photobioreactor and control of parameters such as instrumental and electrical analyses sensors are important considerations for design too.[157]

Different configurations of bioreactor have been designed in the literature, each with varying ability, dependent on algae species and reactor design, to efficiently control process parameters for optimal algae growth. The different factors which impact upon the cultivation of algae in enclosed reactor systems are highlighted in Figure 1:8. Various

types of reactor for algae growth are detailed in Table 1:6, emphasising each configurations strengths and weaknesses. Photobioreactors are designed to increase areal production rates due to optimisation of solar energy, carbon dioxide transfer, mixing and harvesting.[33, 108]

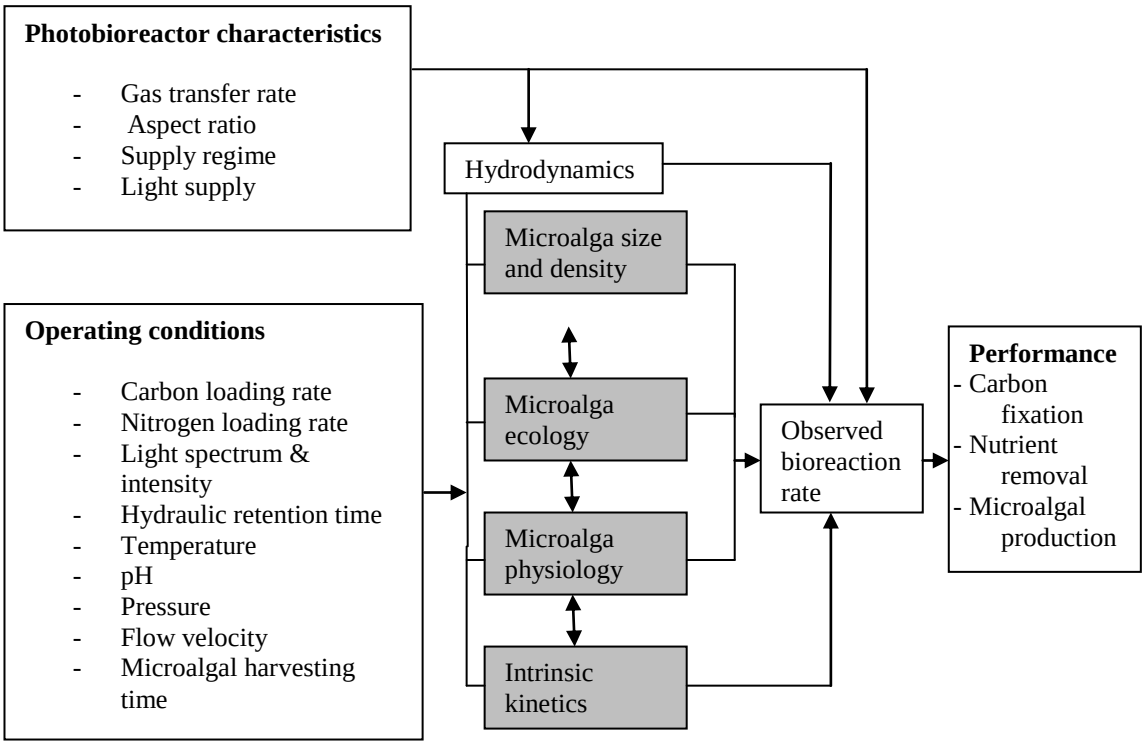


Figure 1:8 – Impact of factors on photobioreactor performance [157]

Fulke *et al.*[101] constructed a 3 L experimental airlift photobioreactor with six 40 W fluorescent lamps which provided a light cycle of 16: 8 h day: night. Filtered air and pure carbon dioxide cylinders were used to aerate the photobioreactor at a rate of 1 L min^{-1} via Teflon tubing. The carbon dioxide and air mixture did require some adjustment using three rotameters to measure flow rates to achieve 0.33 vvm (volume of gas per volume of mixture per minute) of carbon dioxide.[101] *Chlorella* spp. were concluded to have the highest growth rate in this reactor set-up, with maximal growth and lipid productivity observed at 3 % carbon dioxide.[101]

A tubular photobioreactor was initially chosen for this research due to the larger illuminated area, reduced contamination issues and potential for homogenous algal growth; however this was altered to a column photobioreactor to enable successful cell proliferation.

Table 1:6 – Reactor types: advantages and disadvantages[157-158]

Reactor type	Advantages	Limitations
Raceway pond	Relatively cheap Easy to clean Low energy input Easy to maintain Good for mass cultivation	Poor biomass productivity Large area of land required Limited to a few strains of algae Easy culture contamination Inefficient mixing Poor carbon dioxide utilisation Low longevity of algae culture Small illumination surface area Temperature control difficult Scale up challenging
Tubular photobioreactor	Large illumination surface area Low contamination/sterility issues Good biomass productivities Temperature control possible Uniform mixing achievable Scale-up reasonably simple	Degree of wall growth/fouling Requires large land space pH, dissolved O_2 and carbon dioxide gradients along tubes Inefficient mixing
Flat plate reactor	High biomass productivities Low contamination/sterility issues Low O_2 build-up Large illumination surface area Temperature control possible Good gas exchange possible Uniform mixing achievable	Difficult to scale up Degree of wall growth/fouling Small degree of hydrodynamic stress

Reactor type	Advantages	Limitations
Column photobioreactor	Compact High mass transfer Good mixing with low shear stress Low contamination/sterility issues Temperature control possible Reduced photoinhibition and photo-oxidation High potential for scalability Good gas exchange possible Uniform mixing achievable	Smaller illumination area Expensive compared to open ponds Sophisticated construction Decrease of illuminated surface area upon scale-up

1.5.2 Algal reactor types

The best average annual productivity of open ponds (see Table 1:6: Raceway pond) was reported to be with the algae having a lipid content of around 40 % and a specific growth rate ~2 d^{-1}.[159] Considerations for use of an open pond are land costs involved, costs and ease of harvesting the algae, the volume/density of the algae produced and the reliability of sunlight for photosynthesis. Up to 25 % of algal growth can be lost each night due to the respiration of algae cells. Competing species, including bacteria and algae, could easily invade and out-compete algae in open ponds.[160]

Photobioreactors (such as Table 1:6: Tubular photobioreactor, Flat plate reactor and Column photobioreactor) are used to produce algae of a specifically selected strain which has minimal chance of contamination by algal grazers (*e.g.* rotifers), fungi, viruses, protozoa and bacteria. Although open pond cultures of algae are economically more favourable due to low initial set up costs, the closed culture environment is better protected from invading organisms, including competing algae species, and growth parameters can be controlled.[160] With closed photobioreactors less land is required, water is used more efficiently and climate conditions do not affect the growth. Temperature can be controlled, as can carbon dioxide concentration and nutrient levels.[2] A simple 300 L culturing bioreactor system can have a productivity of 100 g dw m^{-2} d^{-1}.[159, 161]

The most popular choices of closed reactors are tubular and flat plate, due to cost and free, readily available light source at reactor locations.[108] Such reactors maximise the surface area to volume ratio as well as the working volume, mixing and carbon dioxide provision.[108] Sensitive reactor design is employed to prevent algae cell shearing. There are three main types of tubular reactor constructed: (i) simple airlift and bubble column which has transparent vertical tubing and bubbling carbon dioxide; (ii) horizontal tubular reactor produced of transparent horizontal tubing often with gas transfer systems attached; and (iii) helical tubular reactors formed from flexible transparent tubing coiled into a circular framework.[108] α-shaped tubular reactors have also been developed which have unidirectional flow which has a high liquid flow rate at the same time as having a slow gaseous flow rate; this design also gives an optimal angle to capture sunlight. Flat plate reactors aim to make maximum use of sunlight with thin panels to provide high surface area to biomass volume ratio.[108] Some designs have open tops to allow the natural release of oxygen into the atmosphere, whilst others are completely closed for maximum contamination control.[108]

Airlift reactors have been used in a variety of applications such as the food and beverage industry, for pharmaceutical and cosmetic production and fine chemical manufacture. The gentle movement of the algae biomass leaves cells intact, and delivers carbon dioxide to the cells whilst minimising oxygen build up. It is also a relatively inexpensive, low energy technique to mix the algae growth medium and deliver gas. Zimmerman *et al.*[162] constructed an airlift looped bioreactor to cultivate *Dunaliella salina* whereupon it was observed that microbubbles (diameter: 500 μm) and fine bubbles (1 – 2 mm) both supplied adequate carbon dioxide saturation of the growth medium. The growth rate was around 30 % higher for the microbubble grown algae, when grown under identical condition.[162] Due to this observation of enhanced growth with smaller bubble size, it was concluded that the reduced bubble size allows for superior algal suspension which allows the algae to remain in the growth medium and proliferate further.[162] Alternatively it has been postulated that the microbubbles remove the oxygen away from respiring algae which eliminates the inhibition of oxygen on algae growth owing to dissolved oxygen build-up.[162]

The use of airlift systems usually results in less shearing of algal cells than mechanical mixing.[108] Airlift mixing can be more costly energetically than mechanical mixing, yet when carbon dioxide is sequestered concurrently the energy costs are balanced.[163] Due to

the absence of moving parts and a simpler construction there is less likely to be a technical malfunction with the airlift system.[108] Minimal build-up of algae onto reactor sections is expected due to their relatively smaller size. The algae can be kept in suspension and build-up of pH, nutrient or temperature gradients can be avoided in an airlift system. The airlift system can provide the photobioreactor with the required supply of carbon dioxide and thus improve the exchange of gases (oxygen and carbon dioxide). Proper mixing may significantly increase the bioproductivity of the algal cells.[164] The cell fragility, density and growth alongside the fluid dynamics and hydrodynamic stress on the algae requires consideration, as does gas exchange and mass transfer of nutrients and algae.[29c] The light intensity and light cycle that the algae are exposed to is critical, as is gas bubble size and distribution in the airlift system. Water quality, salinity, pH, gas exchange, carbon and mineral regulation and availability can all be limiting factors in the growth of algae and need careful consideration and analyses.[29c]

The advantages of an enclosed airlift membrane system are that (i) little carbon dioxide is lost to the atmosphere, (ii) there can be accurate control of the carbon dioxide rates and (iii) higher concentrations of carbon dioxide can be used as it is not in direct contact with the liquid biomass. When compared to bubbling the overall efficiencies of the airlift membrane system are improved (25 – 65 % *cf.* 13 – 20 %).[108] The disadvantage of airlift membrane system is that a large membrane area is required to maximise gas transfer. Also the pressures inside the membrane which are necessary to ensure gas transfer are high, causing costs to increase.[108] At high pressure, expansion of the membrane can occur which can cause microspacing leading to bacteria growth in the spaces. This reduces the contact area between membrane and liquid biomass thus decreasing transfer rate over time.[108] The use of a bundle of microporous hollow-fibre membranes to supply carbon dioxide can reduce the required low pressure due to a lack of hydrostatics.[108]

1.5.3 Examples of photobioreactors

Lu *et al.*[133] report that in outdoor closed reactors the biomass productivity for helical reactors is 0.54 g L^{-1} d^{-1}, whilst for a bubble column it is 0.16 g L^{-1} d^{-1}. The mean daily irradiance at the culture surface, during daylight, was calculated as a fraction of solar irradiance on each reactor surface at different hours (I_s/I_o). The mean daily irradiance for the helical reactor the I_s/I_o ratio was 0.38 and for the bubble reactor it was 0.42.[133] This indicates that there is higher light intensity at the surface of the bubble reactor. The helical

reactor has a larger surface area and therefore the algae has higher exposure/availability and reduced light path length. For the helical reactor the biomass productivity increased with airflow rate despite a decrease in solar irradiance.[133] Chlorophyll fluorescence values for the strain *Monodus subterraneus* in both reactors were lower than those measured in indoor conditions. The influence of dilution rate, air flow rate and solar irradiance on growth rate and biochemical composition was analysed, along with photoinhibition and photo-oxidation.[133]

A bubble column photobioreactor[165] has been used outside for batch analyses of various algal species. The maximum biomass productivity obtained using this photobioreactor at exponential growth phase was 1.840 g L^{-1} day^{-1} for *Chlorella* spp. and 1.667 g L^{-1} day^{-1} for *M. subterraneus*.[165] These results are higher than the 0.68 g L^{-1} day^{-1} obtained for *Chlorella spp.* biomass cultured using a cone-shaped helical tubular photobioreactors.[166] As for all algal cultures the results are highly species specific, however general trends may be transferable.

Continuous microalgae production by a 120 L prototype photobioreactor is being used in a French mollusc hatchery.[167] The specifics of the hatcheries have forced the design to become a closed artificially illuminated system with an external loop airlift configuration. It has two transparent vertical interconnected columns produced from transparent polymethylmethacrylate (PMMA) which allows effective light penetration and clear visual observation. This section is composed of two vertical columns, a riser and a downcomer which are connected by two flanges.[167] Circulation of the algal mixture is induced by a hydrostatic imbalance due to the density difference between the gassed and ungassed liquid columns. There is also pneumatic circulation of the liquid; though this has a very low superficial gas velocity (<1 cm s^{-1}).[167] The airflow, which was determined by overall volumetric mass transfer coefficient measurements, enabled the system to remove oxygen produced photosynthetically. Loubiere *et al.*[167] noted that biofouling on the reactor walls was significantly reduced due to the airlift system.

1.5.4 Mixing

Mixing is a key component of algae growth leading to increased productivity postulated to be due to increased frequency of cell light/dark cycling and improved mass transfer between nutrients and cells.[168] *Chlorella vulgaris* growth is increased with improved

agitation due to better aeration.[15] Mixing is critical to ensure homogenous growth in all areas of the reactor.[108] This is related to gas transfer, and includes temperature hot and cold spots as well as shading of the algae cells. A uniform dispersion of algae is important for optimal growth as it improves productivity by increasing cell exposure to light whilst enabling mass transfer between nutrients and cells. Mixing of the microalgae, nutrients and medium is integral to maximising the growth rate of the microalgae due to optimal light saturation of the algae cells, optimal light intensity for algae is 5 – 10 times less than average solar light intensity.[108] As algae cultures replicate and biomass increases the light gradients become steeper and light availability per algal cell decreases. The ideal is a homogenous mixture where all algae cells spend a similar amount of time in the light path, thus being able to optimally photosynthesise and replicate.[108] The dark periods experienced by the algae cells should increase growth rate due to photosynthetic efficiency and less photoinhibition; as unused photons disperse energy as heat.[169] Thermal gradients of up to 8 °C have been recorded between top and bottom of unmixed bioreactors. This leads to irreversible damage of the algal cells.[170]

The rate of mixing of the microalgae affects the light intensity as in dark areas photosynthesis cannot occur leading to lower bioproductivity.[108] To ensure higher bioproductivity vigorous mixing is essential and the density of the algae must be considered as when algal biomass is dense the light penetration is reduced to 2 – 5 cm.[171] Nedbal *et al.*[172] report that whilst "uniform bubble lifetime is hard to achieve because of the statistical bubble radius dispersion and frequently uneven flow rates of bubbles" the size of bubbles does not affect carbon dioxide transfer significantly. Smaller gas bubbles lead to more efficient and less turbulent mixing, increasing homogenous algal culture growth.[173] Mixing of liquid biomass can be random and lead to stagnant zones where anaerobic conditions hinder algae growth.[108] Dead zones encourage the growth of toxic species which contaminate the whole bioreactor. The increased oxygen levels can lead to reversible destruction of components of the photosystem II, which can lead to a reduction in yield of biomass.[174]

As recorded earlier (see section 1.5.2) there are two main ways to mix bioreactors, one is mechanical and the other using the air input; there are also other techniques. It has been reported that the centrifugal mixing and rotary positive displacement pumps cause a drop in bioproductivity; proportional to their rotation speeds.[108] Mechanical mixing can cause

shearing due to high liquid velocities, turbulence of the culture and the hydrodynamic stress induced. This mechanical stress can be dampened by promoting less aggressive but entirely adequate mixing created from the use of baffles to control turbulence.[175] Gas mixing systems cause less shear stress damage to algae cells reducing hydrodynamic stress and forming good turbulence, particularly airlift systems where fluid flow obtained from sparging air into a central draught riser causes a decrease in bulk liquid density, making it rise gently.[108] However issues with bubbling can include biofouling which obstructs gas transfer and requires cleaning as well as the loss of carbon dioxide to the atmosphere due to low residency time of the carbon dioxide in the liquid biomass.[108] In the airlift system the air bubbles are used to move the algae; this can cause damage to the microalgae as the algae cells stick to the air bubbles. The damage can be reduced by adding a non-ionic surfactant such as Pluronic F-68 for animal cell cultures[176] and for microalgae if shearing is prevalent.[177] Carboxymethyl cellulose may also reduce shear stress on algae samples.[178] This shearing can be counteracted by maintaining effective but low gas entry into the sparger, removing the requirement for surfactant addition.[33] The use of airlift mixing can strip off oxygen from the water which improves the photosynthetic rate of the algae; this is intrinsically linked to temperature and a decrease in temperature can cause significant increase in oxygen levels and hence a reduction in photosynthetic rates.[175, 179]

1.5.5 Carbon dioxide supply and control

The form of carbon the algae uptakes is not of critical importance as this is not a limiting step in the growth of algae. Some algae uptake aqueous carbon dioxide with others preferring to transport bicarbonate releasing carbon dioxide.[172] The algae can utilise atmospheric carbon dioxide, that from flue gases or chemically fixed carbon in the form of inorganic carbonates.[33] Inorganic carbon affinity at the cell surface for *Chlorella vulgaris*, was found to be very low (<1 μg carbon L^{-1}) and the synthesis of carbonic anhydrase is enhanced at low carbon dioxide concentration.[108] It is considered that 30 ppm of carbon dioxide is required on the surface of algal cells to maintain photosynthesis rates. However, experimental evidence suggests that after 100 m path the algae has used up all carbon dioxide supplied and so requires more for continuation of growth thus carbon dioxide must be readily available.[108]

Studies have been carried out which limit carbon supply to study the relationship between bubble size, partial pressure of carbon dioxide and gas flow rate.[67] High bubbling rate with

small bubble size and low input of carbon dioxide was found to give optimal biomass production (47 % more efficient), as did conditions of low bubbling rate with high input of carbon dioxide (14 % more efficient). When the bubbling rate was high, cells flocculated easily.[108] With input of 1 % carbon dioxide productivity was independent of bubble size which is thought to be due to the partial pressure in the culture being high enough to achieve limitations of light. Conclusions of these tests were that total carbon availability is determined by both gas bubbling rate and partial pressure of carbon dioxide.[108]

In open ponds the normal air concentration of carbon dioxide is 0.03 %, thus the carbon transfer to the algae cells is minimal. If the carbon dioxide levels are increased this can force light to become the limiting factor in the growth of the cells.[108]

When carbon dioxide is supplied to algae cells for growth in the culture medium it dissolves in water to become hydrogen carbonate (HCO_3^-) which is then absorbed by the algae for photosynthesis.[164] Transfer rate of carbon dioxide into the culture medium depends on many factors such as pH, temperature and interface proportion of liquid and gas, thus it is important to supply the carbon dioxide in an appropriate way.[164] The regulation of pH can be used to control the amount of carbon dioxide supplied to the algae system. Carbon dioxide is frequently used in literature to regulate pH by injection into the growth medium when pH increases.[180] Though an effective method of pH control it has slow response times due to dispersion throughout the growth medium and chemical equilibrium being reached. Alternatively a model-based predictive control can be used to predict the process outputs within certain time frames; sampling can also occur and is used to improve the predictions.[180]

1.5.6 Oxygen saturation

Light intensity is important in the photosynthetic part of algae growth; however oxygen can be a limiting factor for photosynthesis as it is a reversible reaction (Figure 1:9).[108] The oxygen can lead to photo-bleaching of the algae and reduce the photosynthetic efficiency of the photosystems.[181]

$$\textbf{Energy} + CO_2 + H_2O \leftrightarrow \textbf{sugar} + O_2$$

Figure 1:9 – Photosynthesis[108]

Oxygen concentrations above air saturation inhibit photosynthesis in algae growth and can become toxic (>35 mg L^{-1}) when dissolved in the liquid biomass.[108] With small bioreactors poor mixing and inadequate gas transfer methods are adequate; however as the size of the bioreactor increases oxygen inhibition can increase exponentially.[108] This was highlighted in the failures of a commercial device which was several kilometres long.[182] Accumulation of oxygen is a particular issue in horizontal tubular reactors which have a high area to volume ratio; usually a degasser is used to remove this excess oxygen. Alternatively vertical alveolar panels have been designed where carbon dioxide enriched air is sparged at the bottom and oxygen comes out of the liquid biomass at the top.[183]

1.5.7 Mass flow control meter

Mass flow controllers provide accurate measurement and can be used to control gas flow independent of flow pressure change and temperature within a given range whereas mass flow meters give an accurate measurement of gas without controlling the flow rate.[184]

To ensure the accuracy of gas flow measurement clean, filtered gases, such as bottled carbon dioxide or compressed filtered air, should be used to avoid clogging of the systems.[184-185] The mass flow controllers are gas specific and generally attached in series to the reactor.

1.5.8 Cleaning

Cleaning the fouling on the photobioreactor is integral to ensuring optimised running of the system. Accumulation of dirt on the internal pipes and connectors as well as on the outside of the reactor can exclude light from the illuminated sections of the photobioreactor and affect growth rates. Contamination with pathogens and biological competitors are a potential threat to healthy algae growth. There are different methods available for the disinfection and sterilisation of the photobioreactor. In literature washing once with deionised water containing 0.04 % NaOCl and 0.2 % NaOH then three times with deionised water was employed.[186] Alternatively 1 wt% NaOH solution was circulated for 15 – 20 minutes.[71]

1.5.9 Light requirement and light emitting diodes

Phototrophic algae require light to grow and can use any form of light within the right wavelength parameters (see section 1.5.15). There are many different kinds of illumination

including natural light, fluorescent tubing, high pressure sodium lamps, metal halide lights and LEDs each with individual advantages and disadvantages. Recent improvements in semiconductor technology and light emitting diode (LED) manufacture allows research into semiconductors as a light source using converted solar radiation. This means that the photons can be tailored to have specific characteristics optimised for algae growth. The use of ultra-efficient high-flux photovoltaics is required to convert the solar radiation into electricity. Subsequently the use of LEDs to convert the electricity into pulsed, monochromatic, red or blue light can more efficiently use the white light captured.[36a] Matthijs *et al.*[187] reported that the use of flashing LEDs in indoor cultivation was preferable to the use of luminescent light sources.

The lifetime of LEDs is of the order 100 000 h, which means that replacement of the LEDs would be required, on average, every 11.5 years which is much less frequently than routine maintenance checks would be required on industrial scale machinery.[36a] Solar cell efficiencies have increased to over 40 % and high flux fibre optics are around 85 % efficient, thus giving systems which have efficiencies of approximately 34 %.[36a] Pulsed LEDs can convert 60 % electricity into red light and allowing for irradiance levels at the photobioreactor surface to range from normal solar intensities (averaging around 350 μmol photons m^{-2} s^{-1}) to around 100 000 μmol photons m^{-2} s^{-1}.[36a] The photovoltaic–LED combination can transform the solar energy into pulsed red light with around 20 % efficiency using commercially available components. The energetic loss due to the conversion is compensated by the 500 % photosynthetic advantage of producing solely red light which is about five times as effective as a full-spectrum solar energy.[36a]

The main issue with using LEDs and converting the solar radiation into pulsed light is the heat energy which is created though this is reduced compared to conventional lighting. This extra heat could have a detrimental effect on the algae growth. Thus cooling, most easily by water, is required of the system as well as LED heat sinks which can be located externally to the photobioreactor. Maximum operational currents of the LEDs are 155 mA when pulsed with a minimum of a 0.1 ms gap; otherwise overheating and malfunction of the LEDs occurs. Continuous LED use can have a maximum current of 30 mA.[188]

The use of LEDs continuously is also advantageous since algae cells undergo loss of cell mass during simulated or real ‘night periods’.[189] The use of LEDs has also been shown to

successfully cultivate algae cells; total biomass production was unaffected though the average cell volume decreased to half the volume when fluorescent light was used for growth.[190] However, if red LED light is used prior to fluorescent light, the increased number of algae cells can increase in size when exposed to fluorescent light, hence increasing overall biomass.[190]

1.5.10 Photosynthesis

Light is critical to the growth of algae species as it provides energy which can be transformed by photosynthesis. Photosynthesis is the process by which solar radiation is used to produce carbohydrates and energy for plants (Figure 1:9).[92]

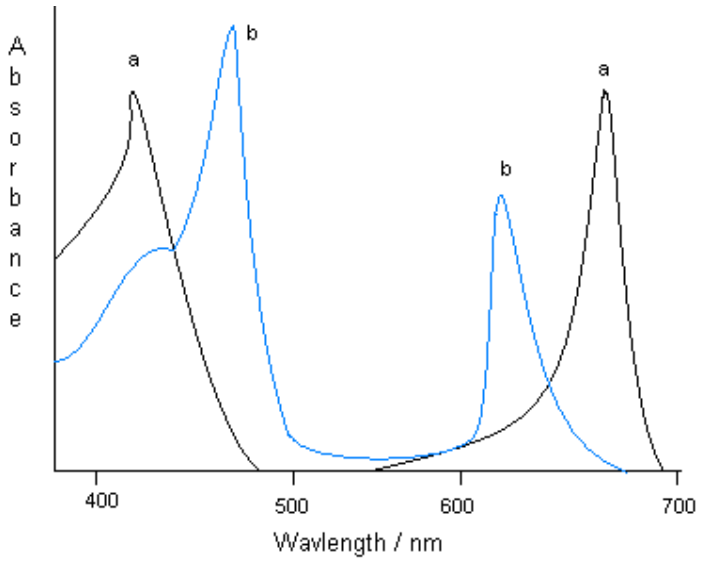


Figure 1:10 – Absorption spectrum of chlorophyll[105a]

Chlorophylls, which are found in algae as well as plants, absorb in the blue and red parts of the spectrum (Figure 1:10). Light of wavelength 600 – 700 nm generally has high efficiency for photosynthesis.[104b] The concurrent use of wavelengths between 400 – 500 nm has been shown, in some cases, to increase the overall rate of growth of plants. It is rationalised that the light serves another wavelength dependent purpose than solely photosynthesis.[104b] It has been suggested that algae require 5 – 10 % blue light for other metabolic functions.[93a, 140] Engelmann showed in 1884 that white light can be split into its spectral components using a prism.[36a] Green algae can be illuminated with only the blue and red parts of the spectrum and photosynthesise in a normal manner. This is shown in the absorption spectra of the pigments chlorophyll *a* and *b* in Figure 1:10. Blue photons have 7/4th the energy of red photons. Both red and blue photons can displace electrons in the

reaction centre of chlorophyll; extra energy from shorter wavelength photons is dissipated as heat which is photochemically inefficient.[191]

Of the 1370 W m^{-2} of light energy that reaches the outer atmosphere of the earth only an average of 240 W m^{-2} reaches the earth's surface.[105b] A fraction of this amount fixes carbon dioxide via photosynthesis with efficiencies of 0.1 – 8 % for total irradiance. Theoretical maximum quantum efficiency (maximum capacity for photochemical energy) of carbon fixation is just 0.125 mol C (mol quanta)$^{-1}$ which is equivalent to maximum productivity of around 29.8 g dw m^{-2} day^{-1}.[105b] This could be increased to a maximum of 200 g dw m^{-2} day^{-1} in intermittent high frequency light (around 8 times higher than the average measured under field conditions, when 25 g dw m^{-2} day^{-1} is considered high).[105b] According to literature, higher yields and photosynthetic efficiencies can be achieved by limiting antennae size or pulsing light at frequencies equivalent to the electron turnover in the electron transport chains within the photosynthetic process.[105b] It is thought that the scaling up of reactions and the non-optimisation of their conditions is the main issue to the reported bioproductivity rates.[105b] In tanks *Chlorella* spp. have shown photosynthetic efficiencies of up to 15 % of incident light, in open ponds in summer efficiencies of between 2 – 3 % have been achieved.[105b] The lower the intensity of light the higher the photosynthetic efficiencies obtained.[105b] It is known that, dependent on wavelength numbers, between 8 and 12 photons are required for the fixation of one carbon dioxide molecule and the evolution of one oxygen molecule. It is generally accepted that the maximum quantum yield (Φ_{max}) is 8 photons. This suggests that there must be an upper limit for carbon fixation dependent on this value, but this is currently not the rate limiting part of the algae productivity.[105b]

The natural diurnal cycle and seasonal variation of temperature and light availability can present issues for algae cultivation.[13] Whilst natural light is freely available the commercial application of it, particularly in Northern Europe and the UK, is limited.[13] The degree of control for temperature and light intensity, wavelength and photo-period duration is more easily controlled in a closed system, though this increases capital and running costs of algae growth.[102a]

Photosynthesis is a two part process and requires electrons for reduction of carbon dioxide to organic material. Phase I (light reactions) is light dependent requiring light energy to

form energy carrier (ATP) molecules which are used in the second phase.[91a] Phase II (dark reactions) is light independent and occurs when products of phase I are used to form C-C covalent bonds of carbohydrates; sometimes called the carbon or Calvin cycle.[108] Evidence suggests that both the light and dark phases are integral to the photosynthesis process to avoid excessive illumination and allow for photon processing.[91a, 192]

The light energy available to each cell is dependent on multiple factors including the intensity of the light, wavelength of the light, density of algae and light penetration due to contaminants.[168] The best way to describe the light distribution in photobioreactors is currently thought to be by diffused light distribution models. Diffused light distribution models take into account the geometry of the photobioreactor, light source, absorption of light by pigments and scattering of light by cells and other particles, thus reducing photoinhibition.[193] It is, however, difficult to predict the light intensity and availability in photobioreactors as the bioproductivity rate can constantly change. The volumetric productivity of algae can be plotted against the optical depth and as such a first order polynomial equation[105b, 194] can be fitted to describe the relationship between volumetric productivity and the optical proximity.[127] It has been reported in literature that there are marked increases in productivity at optical depths of less than 100 mm.[105b]

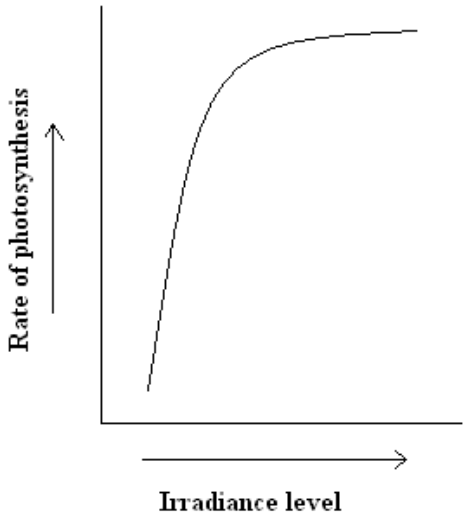


Figure 1:11 – Photosynthesis-Irradiance curve

To predict the growth of an algae culture the relationship between increase in biomass and amount of light received must be analysed – this is the photosynthesis-irradiance relationship (also known as the P-I curve - Figure 1:11). There have been studies of the P-I relationship in numerous studies.[193, 195] However these are empirically measured and do not

take into account the photoadaptive responses of photosynthesis which occur in biological species. One of these photoadaptive responses is that in low light intensities there is an increase in the concentration of light absorbing pigment (chlorophyll) in the cells.[35, 72] To improve on these empirical methods, dynamic models have been developed which breakdown photosynthesis into its different parts including at least one photochemical energy capture step and one metabolic consumption step.[35]

1.5.11 Effect of light on algae growth and productivity

Light availability affects the growth of phototrophically grown algae cells. If there is oversaturation of light growth inhibition occurs, due to dissipation of photonic energy as heat and photoinhibition (see section 1.5.12) of intracellular functions.[36a] For most photobioreactors the optimal photon flux density is 345 – 1125 μmol photon m^{-2} s^{-1}; yet it has been projected that with the use of pulsed light this limit can be improved.[36a] Different light intensities, wavelengths and also the effect of light and dark ratios and frequencies can affect how the algae develop and reproduce.[36a]

Xue[196] has identified four general distinct regions of light intensity: (i) the light limited region – where a lack of light limits the growth of algae; (ii) the intermediate region – where light is at an increasingly optimal level for growth; (iii) the light saturation region – where the algae photosynthetic stages are fully saturated by light, yet not limited by the light; and (iv) the light inhibition zone – where oversaturation of light causes a decrease in growth rate of algal cells.[196] Generally the four zones will be found within a photobioreactor or open pond, with increased light intensity providing increased growth rates at higher light frequency and at increased intensities a lower light fraction being required.[196]

If the dark reactions are the rate limiting process then the photonic flux tolerance and hence the bioproductivity rely on the dark reactions for photosynthesis.[36a] When the time pattern, spectrum and instantaneous intensity of pulsed LEDs are matched correctly to the dark reaction cycle the flux tolerance will be increased. It is postulated in literature that bioproductivity levels of approximately 100 g m^{-2} h^{-1} are possible.[36a] This will require a photobioreactor with thin channels, ultra-dense cultures and rapid light/dark cycles. In literature the intrinsic conversion efficiency of photosynthesis was not increased by the creation of light/dark cycles hydrodynamically.[36a]

Vejrazka at *Wageningen University* in the Netherlands reported that a series of light/dark pulsing experiments were carried out to determine the effect of flashing light on algae growth.[126] The light:dark ratio was kept constant at 0.1; whereas the frequency of flashed light was varied between 1 Hz and 100 Hz. Control experiments were carried out under continuous light. The growth of algae under highly frequent pulsing gave specific growth rates and photosynthetic efficiency as the reference experiment. Comparatively prolonged pulsing of the algae gave a marked decrease in the specific growth rate and consequently a reduced photosynthetic efficiency of the algae.[126] It is hypothesised that the algae may be capable of storing electrons for use during the dark stages. During prolonged light periods the photosystems become oversaturated and a decrease in growth rate is recorded, due to a reduction in efficiency.[126] Periods of low light intensity have also been shown to significantly increase algae growth rate, carbon dioxide uptake and lipid accumulation compared to dark periods.[126] This is countered by Tang *et al.*[125] who reports for *Chlorella minutissima* that as the intensity of light increases from 100 to 200 and onto 350 μE m^{-2} s^{-1} there are significant improvements in growth rate; this plateaus at 350 and 400 μE m^{-2} s^{-1}. The lower the light intensity the more phospholipids and glycolipids are accumulated; whilst at higher light intensity there is a lower percentage of structural and functional cell membrane lipids cultivated.[88] At higher light intensities this study shows an increase in overall lipid accumulation; particularly of saturated triglycerides.[22c, 88] It is also highlighted that different algae absorb light optimally at different wavelengths, and as such, each algal species requires individual optimisation of light conditions and parameters, since light is such an integral growth parameter.[33]

Photoperiod effects are documented for *Chlorella minutissima* that light: dark ratios of 12: 12 and 15: 9 hours have no significant differences. When algal cultures are grown with continuous light slower growth occurs yielding a lower cell density.[125] However, perhaps surprisingly, there was a higher overall biomass harvested with continuous light than the other pulsing regimens. There was no difference between all photoperiods in the percentage of FAME in the biomass or in the FAME profile.[125]

The inefficiency of photosynthesis (8 – 10 % maximum efficiency) in algae has been attributed to chemical pathway hindrance, physical problems or the syntheses of lipids and secondary products.[127] Firstly looking at chemical pathways: (i) photochemical pathways

can be less than efficient in the transfer of excitation energy; (ii) oversaturation of cells perhaps due to the capacity of the Calvin cycle can lead to energy dissipation; (iii) carbon fixation can require more photo-energy than the calculated value under non-optimal conditions; and (iv) during photorespiration carbon dioxide can be outcompeted by oxygen leading to energy losses these losses are lower in algae than terrestrial plants.[127] The physical issues which cause algae to be less efficient can be attributed to the sunlight being unable to be absorbed by the species due to shading in over-dense cultures.[127] Another reason for inefficient use of light is because of the solar energy being of unusable wavelengths.[197]

Algal species yield lower solar energy conversions than devices such as photovoltaic cells. The low efficiency in photosynthesis is due to the inherent 40 % energy losses for absorbance of blue photons by the chlorophyll; this is due to their high energy of which excess is converted to heat.[198] It is proposed that the losses of natural photosynthesis can be attributed to the factors below.

1. The inefficiencies of light harvesting due to enzyme concentration required at different light levels and associated chemical inefficiencies.[199]
2. The shading of chlorophyll pigments algal shading.[198]
3. The photorespiration and respiration occurring in algae.[198]
4. The dependence on a chlorophyll based system which is specifically dependent on harvesting solar energy from the first excited singlet state of chlorophyll α.[199]
5. The non-photochemical quenching of the excited singlet state.[198]
6. The losses due to conversion of carbon dioxide, ATP and NADPH into sugars.[198]
7. The use of two photosystems in series, instead of a single photosystem.[199]
8. Photosystem II is damaged when oversaturation and hence photoinhibition occurs.[199]

Altogether these inherent losses of photo-energy lead to theoretical biomass synthesis yields of 4.5 % for C_3 and 6 % for C_4 plants, whilst observed yield of plant biomass is much lower than this value.[198-199] Light can influence photosynthetic rates of algae in various ways, including, the availability of the photo-radiation, the light history and

acclimatisation of the algae (either continuous or pulsed light) and the frequency and/or ratio of pulsing.[59b]

1.5.12 Photoinhibition in photosynthesis

Photoinhibition is when there is an increase of incident light to the algae which is followed by a decrease of the specific growth rate. Incident light can be difficult to control, especially in open conditions as geographical and climatic conditions can change dramatically over relatively short periods of time.[4] The angle of the incoming light also affects its penetration and influence on the algae; this is continually altering for natural light as the earth rotates around the sun.[4]

It has been reported that algae can become light saturated at reasonably low natural light levels, around 10 % of full sunlight. When there are dark/light cycles the algae can assimilate more light than when continuous 24 hour light is available. The nutrient levels of the growth medium can also affect the light saturation levels due to nutrient starvation reducing the chlorophyll content of the algae.[200] J.S. Burlew stated in the 1950's that the "success of high algae production requires a system where algae can use strong solar light in mid-day as effectively as they use low photon flux in the early morning".[201]

Photoinhibition is biochemically caused by the oxidation of water on the lumen side of the photosystem II and is critical to photosynthesis.[104a] The electrons from the water are light-driven through to NADP creating a proton gradient to facilitate ATP synthesis. This photosystem II is vulnerable to photoinhibition which is stipulated to be a result of singlet oxygen production at the reaction centre causing irreversible oxidation of the D1 protein.[102b, 104a, 202] Since this damage occurs at all light intensities the reaction centre is required to be rebuilt every half hour, regardless of light irradiance level. It is considered that the photosynthetic electron transport system minimises the production of the singlet oxygen at the photosystem II, as well as superoxide and hydrogen peroxide production at the reducing side of photosystem I.[102b, 104a] Usually the photosynthetic electron transport system has a rate of repair of the same order as that of the rate of damage, however when there is high light intensity, the high level of photosystem II activity cannot be maintained leading to photoinhibition or oversaturation.[203] One of the most effective strategies adopted by the photosystem II to dissipate excitation energy of the chlorophyll a molecules is non-photochemical quenching which dispels the energy as heat.[104a, 204] Under high light

conditions, acclimatised algae have high dark respiration rates and thus flourish under high frequency light/dark cycles.[104b]

The reduction of reactive oxidation species production during photosynthesis is carried out by antioxidants, such as ascorbate, glutathione (GSH) and α-tocopherol.[104a] Photosynthesis does, however, require these reactive oxidation species for intracellular redox homeostasis and redox signalling.[104a] The photosystem II reaction has an adequate redox potential for abstracting electrons from water of up to 1.2 V.[205]

1.5.13 Light regimen effects

The application by Ihnken *et al.* of short and long duration light curves (pulsing regimens) to *Chlorella emersonii* highlighted that algae can adapt to different light environments and change photo-physiological response state during steady light conditions.[206] If the algae was acclimatised with dark periods prior to the light curve application this lowered the maximal relative electron transport rates. However after a longer exposure to particular light curves, the algae growth acclimatised.[206] It is suggested that activation states of photosynthetic and photo-protective mechanisms within algae photosynthetic apparatus can be varied and that these systems are highly adaptable. Along with other species analysed, it can be concluded that the response to different light regimens and photon flux is species specific.[206] Clearly under phototrophic conditions light intensity is critical to cell multiplication and lipid accumulation.[92]

1.5.14 Effect of pulsed light on algal growth and acclimatisation

It has been demonstrated that the microalgae does not require continuous illumination to increase in biomass, and that intermittent light of the same optimum intensity can efficiently and effectively promote growth.[207] This pulsing effect is postulated to overcome photoinhibition and oversaturation allowing maintenance of increased specific growth rates. The first reported observation of the flashing light phenomenon for photosynthesis was by Kok in 1953.[201] Following this Richmond and Vonshak[208] in 1978 obtained increased *Spirulina* sp. growth rates due to increased turbulence and the subsequent fast light/dark cycles formed. Terry reported in 1986 that the use of intense light frequencies around 1 Hz increased the biomass concentrations for most algae cultures.[209] For pulse rates between 0.01 and 1 Hz there has been conflicting results of the impact on growth rate; this variance in results is largely due to species differentiation.[104b, 201, 208-209] Optimal photobioreactors

combine rapid algae transit times with ultra-dense cultures such that the incident light is completely absorbed using an optimally pulsed light source.[36a] The use of a pulsed light source to increase the bioproductivity of the algae according to a particular time sequence with pulses of tens to hundreds of μs has been reported in literature.[36a] This is thought to significantly increase the averaged photonic intensity whilst postponing photosaturation. Simple pulsing of the light source does not affect the bioproductivity, however optimal pulsing increases bioproductivity, particularly where narrow reactor channels are used. Whilst LED use will increase operating costs compared to direct use of sunlight, it is hypothesised that the gain in bioproductivity will offset this increase in costs. As dark/light fluctuation frequencies are increased, the photosynthetic rates increase exponentially with significant differences between ratios of 0.5 – 2.0.[105b] A multi-layered photobioreactor[210] has been shown to enhance growth by high mixing rates and acclimatisation to the light conditions.

The maximum quantum efficiency of the carbon fixation (photosynthesis) is around 0.125 mol C (mol quanta)$^{-1}$ along with the photosynthetic efficiency of 8 %.[105b] A photobioreactor built by Grobbelaar *et al.* has a path length of 30 mm and can theoretically have an areal productivity of at least 30 g dw m^{-2} day^{-1}.[105b] When high light acclimated algae is separated from the low light acclimated algae in a two compartment photobioreactor there is an increase of 37 % growth compared to a single compartment photobioreactor.[105b] Adaptation of algae to dominant frequencies and pulse patterns of light has been observed elsewhere in the literature; for example Grobbelaar *et al.* who reported acclimatising their algae for up to 72 hours using pulsing light at a higher intensity than that used within the photobioreactor.[104b] Inkhen *et al.* reported that algae took hours or days to acclimatise to pulsed light, due to the time scale of gene expression pathways and processes.[92, 104b, 206]. The increase in efficiency after change in light conditions is noticeable after 6 hours and optimisation occurs over the initial 48 hours.[104b] This gene expression is stipulated by Foyer *et al.* to be related to changes in reactive oxygen species that enhance oxidation and decrease photosystem II activity in high light conditions, hence the time lag in optimisation of algae growth.[104a]

Generally high light acclimated algae have: (i) higher maximum photosynthetic rates; (ii) higher auxiliary pigments (*e.g.* carotenoids) content; (iii) higher I_k values (the transition between light dependent and light saturated photosynthesis); (iv) lower chlorophyll content

per biomass; and (v) maximum photosynthetic efficiencies.[210] Accordingly, low light acclimated algae have lower maximum photosynthetic rates, I_k values and contents of auxiliary pigments as well as higher maximum photosynthetic efficiencies and contents of chlorophyll per biomass.[210] Algae can acclimatise to conditions, including those of average light per cell, thus those in a lower density batch will tend to have high light acclimated properties as they are less shaded in their environment.[210]

The optimal magnitude of the pulsed light as well as the tailoring of the light and dark phases is specific to the photobioreactor used, according to its geometric and flow properties.[36a] It has been reported that if an adequately intense light source is pulsed rapidly and has a optimal t_{exp}/τ_{dark} ratio there is an increase in the average photonic flux (for example to the order of 10 000 μmol photons m^{-2} s^{-1}) before photosaturation occurs.[36a, 211] This noticeably increases the bioproductivity of the algae with maximum productivity recorded at 17 g m^{-2} h^{-1} hypothesised to be due to the average light exposure being on the timescale of the rate-limiting dark reaction.[92] The idea of pulsed light had not been successfully implemented at the time of the Gordon *et al.*[36a] paper due to the photonic flux level being too high above the natural solar level together with the predominant use of continuous (not pulsed) artificial light. This technique seems to be incompatible with outdoor photobioreactor use, as the sole light source is required to be LEDs.

In other studies[36a, 212] it was confirmed that shorter pulse times increased bioproductivity and photosaturation was reached at the order of tens of microseconds. The photon flux acclimatisation of the algae culture determines the light conditions the culture will optimally grow in.[36a]

It is possible that the efficiency of pulsing light in smaller fractions and using higher photon flux densities will increase biomass growth of the algae. However the absolute length of the light period must be very short if a higher photon flux density is to be used to reach the higher photosynthetic efficiencies so that oversaturation does not occur.[213] Oversaturation will cause a bottleneck in the photosynthetic process, thus not allowing each photon to be exploited fully (see section 1.5.12).[36a, 211a] To allow the algae to sustain metabolism during dark periods there are metabolic processes which generate energy independent of light, however more commonly electron disposal reactions are required to alleviate congestion of electrons due to the oversaturation of the algae photosystem II.

Algae have been proven to increase their enzymatic capacity or reducing their photon capturing efficiency.[59b] In commercial algae photobioreactors the flux tolerance is usually around 200 – 400 μmol photons m^{-2} s^{-1}.[36a] This flux tolerance is not an absolute limit and depends on various factors. A flux tolerance of ~2000 μmol photons m^{-2} s^{-1} has been reported though there is nothing fundamental about this figure.[211a] Instantaneous, but discontinuous flux tolerance of 5000 μmol photons m^{-2} s^{-1} without photosaturation has been demonstrated[214] using pulsed light emitting diode experiments. The rate limiting processes are the photosynthetic dark reactions; therefore there must be sufficient dark time so that the photonic input does not lead to photosaturation.[36a] τ_{dark} is orders of magnitude larger than the time scale required for photon absorption, energy transfer and charge separation.[215] The exposure of algae to light is thought to be optimal when it is closest to the time scale for τ_{dark} due to the dark reactions of photosynthesis being rate limiting.[36a, 216]

It has been reported in literature[175] that there are biomass losses at night when using natural solar sources, these losses would be eliminated by having 24 hour illumination from light emitting diodes (LEDs). There is an enhancement of specific growth rates and productivities for the microalgae when there are fluctuations in the light intensity which are faster than 1 s^{-1}.[169, 217] This is due to photosystems within each microalgal cell only being able to absorb photons into electron transport if they have oxidised quinine Q_A and are not already excited. If the photons are in an excess and are absorbed into the photosystem their energy is dissipated as heat or fluorescence. In microalgae it has also been noted that photoinhibition occurs due to damage of protein D1 in photosystem II.[192b] This damage leads to a reduction in the number of active photon traps and thus a decrease the growth rate. [192b] Janssen *et al.*[218] reported that the specific growth rate decreased proportionally to the exposure time of *Chlamydomonas reinhardtii* and *Chlorella sorokiniana* to the light. Hence these algae species are thought to be unable to store light energy to maintain growth under dark conditions.[218] The specific light absorbing surface of *Chlamydomonas reinhardtii* is increased by a doubling of chlorophyll-α content of the algae cells; particularly under light/dark cycles of 13 s at illumination of 240 μmol m^{-2} s^{-1} compared to continuous light. Such changes decreased the biomass yield of the algae species.[218]

If the light intensity is increased the specific growth rate will increase up to a point, after this there is photoinhibition and reversible damage to photosynthesis. This reversible damage may be due to excess photons forming singlet oxygen which oxidises and damages the photosystem II.[219] It is assumed that the photoinhibition has a square root dependency on the irradiation, and that the photosynthesis obeys Michaelis-Menten kinetics.[35]

Other work carried out on cultures of *Dunaliella tertiolecta* where light/dark cycles of 94/94 ms were capable of increasing the photosynthetic efficiency due to a light integration effect.[169] Under 3/3 s cycles and under 31/156 ms cycles the yield was lower than under continuous light. Alternating photoperiods (light) and scotoperiods (dark) under certain conditions can enhance the growth rate compared to continuous light source.[213] Light integration, explained as light energy, can be used more efficiently by the photosynthetic apparatus if delivered in small pulses which are separated by time (rather than a constant flux of light). This can lead to the partial reoxidation of the plastoquinone pool in the photosynthetic membrane during dark periods which leads to an increased capacity to convert light energy to chemical energy during the light pulse.[102b] The changes in the oxidation/reduction state could influence the antenna size of the photosynthetic apparatus. The specific light absorption should be considered when evaluating the impact of light/dark variations.[102b]

In literature[201, 209, 220] the trialled light/dark frequencies have all either been too low, <0.1 Hz, or too high - >100 Hz, except for Grobbelaar *et al.*[104b, 221], Lee *et al.*[222], Phillips *et al.*[223], and Xue[196]. Grobbelaar *et al.*[221] tested pulse widths between 3 and 240 seconds, Lee and Pirt[222] reported using 40 second cycles and Phillips and Myers[223] studied the effect of pulsing light using a chopped beam method to provide pulse widths of 1, 4, 17 and 67 ms with various unquantifiable dark periods. The flashing of 1 ms light was reported to give growth insignificantly different to continuous light, whilst there was a partial advantage of intermittent light for longer pulse widths due to the higher efficiency of light utilisation.[223] Janssen *et al.*[216] reported that with light/dark periods of medium frequency there is an increase in productivity which is dependent on the light fraction of the cycling. The increase in productivity is stipulated to be due to the influence of dark periods on growth rate. The longer dark stages impact growth rate negatively due to the loss of algal biomass over prolonged dark stages (like night periods which, in pulsing studies, are aimed to be eradicated due to their negative effect on biomass accumulation).

Hence the frequencies researched are of integral importance to the rate of photosynthesis and the effectiveness and impact of the pulse rate on growth rate. Medium frequency pulsed light is reported by Xue[196] using high intensity white LEDs with a frequency of 0.01 – 20 Hz, and thus at a lower frequency that the continuous illumination afforded under sunlight. The medium frequency pulsing can lead to improvements in specific growth rate, but it is not guaranteed.[196] It is proved difficult to describe the individual light history of algae cells, since each experiences a unique light pattern whilst in the bulk algae growth medium.[196] The use of flashing cycles impacts the specific growth rate depending on the applied light intensity, yet increasing turbulence in a system requires an increase in energy input which usually counteracts the increased growth rate energetically. Turbulence forms a wide range of fluid residence times and light length ranges, thus it is difficult to observe the effect of particular light experiences of algal cells.[224] With adequate mixing it has been shown that the depth or thickness of the algae layer is irrelevant to the light assimilated by an algae population (see section 1.5.4). There is a significant increase in growth rate with increased mixing rate observed until the maximum growth rate has been reached.[224]

Differences between laboratory scale growth and pilot scale algae growth have been observed by Janssen *et al.*[169] with photosynthetic efficiency as determined by biomass yield per mole of photons absorbed over light/dark cycles of 3/3 s, 94/94 ms and 31/156 ms. The 94/94 ms light/dark increased the photosynthetic efficiency of the algal cells whilst the other light regimens decreased yield compared to continuous light. Janssen *et al.*[169] hypothesise that very short light/dark cycles are required to obtain higher photosynthetic efficiencies (<200 ms) and that either a light exposure of 10 ms or less would be optimal or the use of lowered photon flux density.

Chlorella pyrenoidosa was grown under continuous light and intermittent light of 1, 4, 17 and 67 ms with various dark periods by Phillips *et al.*.[223] It was reported that the 1 ms light pulse width is almost identical to that of continuous light in relation to the light intensity and growth response. For increased pulse widths the similarity of light intensity to growth response remains significant though it lessens with increased length of light period.[223] Whilst there are predicted advantages of intermittent light, caution is exercised over the intermittency phenomenon as it is under-studied and could be attributed to turbulence in algal suspensions.[223]

In summary photosynthetic rates have been shown to increase exponentially with increasing light/dark frequencies depending on the following factors: (i) frequency of light/dark fluctuations[105b]; (ii) light acclimated state of algae (from dark through to high light acclimated algae)[105b]; (iii) ratio of light:dark stages (significant differences were seen between ratios of 0.5 – 2.0)[105b]; and (iv) the light history of the algae (particularly that of algae subjected to continuous light compared to fluctuating light).[105b] Photosynthetic rates increased an average of 2.1 times when equal light/dark phase frequencies were increased from 10 s to 10 ms.[104b, 105b] With increased light phases the photosynthetic rate was not increased, however when dark phases were twice as long as light phases the photosynthetic rate increased by up to 6.7 times.[104b, 105b] Algae grown in low light conditions are reported to have higher rates of photosynthesis at higher frequencies and light/dark ratios of 1:1, though the algae became increasingly efficient in using total light energy when the dark period lengthened.[104b]

1.5.15 Effect of wavelength on algae growth

The effect of wavelength on *Nannochloropsis* sp. grown in phototrophic and mixotrophic conditions has been reported by Das *et al.*.[225] The study showed that continuous illumination had a detrimental effect on algae growth and resulted in a loss of energy as well as reducing cell density.[225] Mixotrophic growth yielded the highest FAME productivity per litre of growth medium, with blue light yielding the highest FAME by colour (55.13 mg L^{-1} phototrophic, 111.96 mg L^{-1} mixotrophic), followed by white light (50.40 mg L^{-1} phototrophic, 87.87 mg L^{-1} mixotrophic). [225] This has been corroborated by Perez-Pazos *et al.*[226] by the optimal yield of lipid in *Chlorella* sp. using blue light and supplementation with $CaCO_3$ and a photoperiod of 18: 6 hours. The blue light is postulated to affect the expression of lipid synthesis genes within the cell nucleus.[226]

The use of blue LEDs to grow *Spirulina platensis* yielded the lowest biomass growth, which was attributed to the absence of absorption bands of chlorophyll. Whereas for red highly intense light increased biomass was cultivated and there was the largest specific growth rate.[227] This was corroborated by Love *et al.* whose research at the *University of Exeter* showed that the use of blue light was ineffective for the growth of green algae, whilst red light was comparable to white light.[227]

It has been reported by Tang *et al.*[125] that fluorescent lights (2.3 x10^8 cells mL^{-1}) are more effective for growth of *Chorella minutissima* when compared with white (1.7 x10^8 cells mL^{-1}) or red (1.7 x10^8 cells mL^{-1}) LEDs. The use of red LEDs led to lower C18(3) proportions, however this does not affect the overall amount of unsaturated FAME. Supplementary carbon dioxide was available to the algal culture. Faster algal growth was achieved by increase of light intensity as well as increasing the period of the light.[125]

A reactor constructed with 128 red homogeneously distributed LEDs in a panel which emitted light in a narrow bandwidth around 637 nm.[228] *Chlorella sorokiniana* was cultured under irradiance conditions of up to 2100 mmol photon m^2 s^{-1} which yielded, under continuous illumination, a maximum productivity of 7.7 g dw m^2 h^{-1}.[228] This maximal productivity was attained at high dilution (0.24 h^{-1}) and low biomass concentration (2.1 g L^{-1}). The high productivity is attributed to the species of algae chosen, *Chlorella sorokiniana*, which tolerates high temperatures and irradiance also having high specific growth rates coupled with the photobioreactor configuration of narrow light path and improved mixing.[228] The discrepancy between observed and theoretical specific growth rate was accredited to the thermal dissipation of excess light energy absorbed.[228]

The growth of *Chlorella* sp. using solely red light by Perez-Pazos *et al.*[226] yielded maximal lipid under conditions of 700 nm wavelength, $CaCO_3$ concentration of 0.5 g L^{-1} and a photoperiod of 6: 18 hours. The short light photoperiod is suggested to be successful for algal growth due to the red light stimulating a higher level of excitement for the chlorophyll electrons which increases the effectiveness of the chlorophyll molecules.[226] There is hypothesised to be sufficient energy within the algae to sustain the growth throughout the 18 hours of darkness for lipid synthesis.[226]

1.5.16 Carbon dioxide capacity and affinity in photosynthesis

Adenosine-5'-triphosphate (ATP) aids the conversion of carbon dioxide into sugar by the use of the enzyme ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBisCO). At least 8 mol of photons are required for the synthesis of each mol CH_2O, giving an average solar energy conversion efficiency of 27 %.[13] RuBisCO has a low affinity for carbon dioxide and at atmospheric levels of carbon dioxide achieves just half saturation of the enzyme which reduces efficiency. The oxygenase activity of the RuBisCO impacts the biomass formation reducing it further by 50 %.[229] Algae has acquired a separate mechanism which

accumulates carbon dioxide at low concentrations to overcome RuBisCO's low affinity for carbon dioxide.[107] The mechanisms, known as CCMs (carbon dioxide concentrating mechanisms), achieve uptake of dissolved inorganic carbon with an efficiency dependent upon species, light, recent history of cells, temperature and pH. Carbonic anhydrase expression, which interconverts between carbon dioxide and HCO_3^- mobilising the carbon dioxide for use by RuBisCO, has been linked with the induction of CCMs.[107]

Jacob-Lopes et al.[230] culturing *Aphanothece microscopica Nägeli* reported that the capacity of carbon dioxide sequestration and oxygen release during day/night experiments was 78 % and 65 % respectively of that achieved during continuous illumination; measured by maximum cellular density. Without the additional organic carbon from wastewater the ratio between the maximum cellular densities was 40 %, this indicates that the organic carbon supports growth in light and dark periods resulting in mixotrophic growth, not just photoautotrophic growth. It was also found that only 3.9 % of total carbon dioxide sequestered was fixed by the algae biomass therefore there must be other routes of conversion available to the carbon dioxide in the photobioreactor such as precipitation of carbonates and bicarbonates facilitated by the increase in alkalinity from algae growth. Other routes such as biological fixation for use in biopolymers and the release of volatile organic compounds could also be responsible for carbon sequestration.

1.6 Summary and aims

As reported in this introduction there is a requirement for renewable energy, particularly for the transport industry. It is suggested that algal biodiesel has the potential, with sufficient development, to meet that need and to bring other positive attributes to the environmental debate. Algal biodiesel can be developed with the necessary research into photobioreactors, to enhance algal cultivation, and with good analysis techniques and biodiesel formation, algae can be one of the key economical and environmental solutions to the 21st century fuel predicament.

The broad aims of this thesis are: (i) to design, construct and test a pulsing LED photobioreactor to increase the efficiency in use of photons by algal cells; (ii) to optimise growth conditions for *Chlorella emersonii* and; (iii) to explore the effects of light and growth conditions on the resultant biodiesel FAME profile.

More specifically the aims of this thesis are: (i) the identification of a relevant algal species for testing and manipulation for algal biodiesel, alongside the determination and application of relevant lipid extraction and transesterification techniques; (ii) the development of accurate qualitative and quantitative FAME profile analysis using GCMS/FID; (iii) the design, construction and testing of a unique photobioreactor including a pulsing bi-chromatic light source to successfully cultivate the chosen algal species to contrast with continuous white light; (iv) to analyse and compare the resultant FAME profile of the chosen algal species grown under different light conditions, varying wavelength at a specific pulse width of 10 ms and pulse length of 30 ms, to enable improved light economy without compromising algal growth or lipid quality; (v) to observe the effect of length of cultivation of the algae and the availability of nutrients to the culture on the FAME profile and lipid amount; (vi) to analyse the synergetic effect of length of cultivation of the algae and the availability of nutrients to the culture on the FAME profile and lipid amount; (vii) to elucidate the carbon requirements of the chosen species of algae; and (viii) to explore the use of microwave technology for the potential lipid extraction and transesterification of algal lipids, whether a one-step extraction is possible and the effect of extraction method on resultant algal FAME profile.

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2 Photobioreactor design, construction and testing

The design and construction of a flashing LED photobioreactor was undertaken to optimise phototrophic algal growth. There are a number of different photobioreactor designs which are summarised in section 1.5.2. An airlift design was used to reduce the shear stress on algal cells and to ensure homogeneous mixing of growth medium, algal cells and gases.

The algae were grown initially for biofuel production in the form of lipid content, however, it was envisaged that this might be a useful starting point from which other product formation can be explored, such as hydrogen production, bioethanol and higher value products in the future.

A closed photobioreactor with a unique pulsing LED light stack has been constructed to exclude algae contamination issues, increase cell density, reduce water and nutrient loss to the environment and to enable a standardised production of algae for biodiesel and other compound production. The photobioreactor could also be used to cultivate sterile and specific inoculation cultures for open ponds in the future. The photobioreactor incorporated an airlift section to provide continuous homogeneous flow of algae biomass and pulsing light emitting diodes (LEDs) to reduce photoinhibition and allow for control and optimisation of light intensity, spectral and temporal output of the photons.[1] The photobioreactor used an internal compressed air system to provide flow and carbon dioxide from a cylinder to supply the carbon required for algae growth, which were controlled by two calibrated mass flow control meters. Both of the gas sources were connected by a t-piece joint then supplied to the culture via one sparger (Figure 2:2). The initially designed photobioreactor was unsuccessful for algal growth, however an "off the shelf" design proved successful for use with the unique light stack. The light stack enabled bi-chromatic pulsed LED light to be compared with continuous white light for algal growth. The resultant FAME profile from the algal lipid was elucidated for each of the successfully grown algal cultures under different light conditions. Both photobioreactors enabled the observation and analysis of lighting effects on 7 L algal cultures which were larger than the 500 mL cultures reported in literature, allowing for lighting implications to be analysed on a larger scale culture.

2.1 Initial design ideas

The initial design (Figure 2:1) for the tubular, airlift photobioreactor included many of the key inputs required such as air, carbon dioxide and fresh media access points. There was also cooling water present, optional pumping systems, a pH meter and thermocouple and vapour condenser which were all incorporated into the final design. The major change which was undertaken from this preliminary design was the disregarding of the falling film photobioreactor section in favour of a concentric tubular design. The initial flat panel reactor design raised major leaking concerns due to the sealant around the edges being exposed and extensive. This was combatted with the tubular design that allowed for the illuminated section to be disassembled from the top down for cleaning of any algae build-up. Preliminary designs of the concentric tubular illuminated section included LEDs mounted on a movable platform outside of the tubes; this would have allowed light intensity to be increased and decreased by proximity to the algae. Subsequent designs called for a less mechanically complex LED mount thus the light intensity was changed electronically by switching on and off of individual LED strips. The photobioreactor design needed to withstand autoclaving for decontamination of all sections in case of bacterial or fungal infection, or algal species competition (see section 2.2).

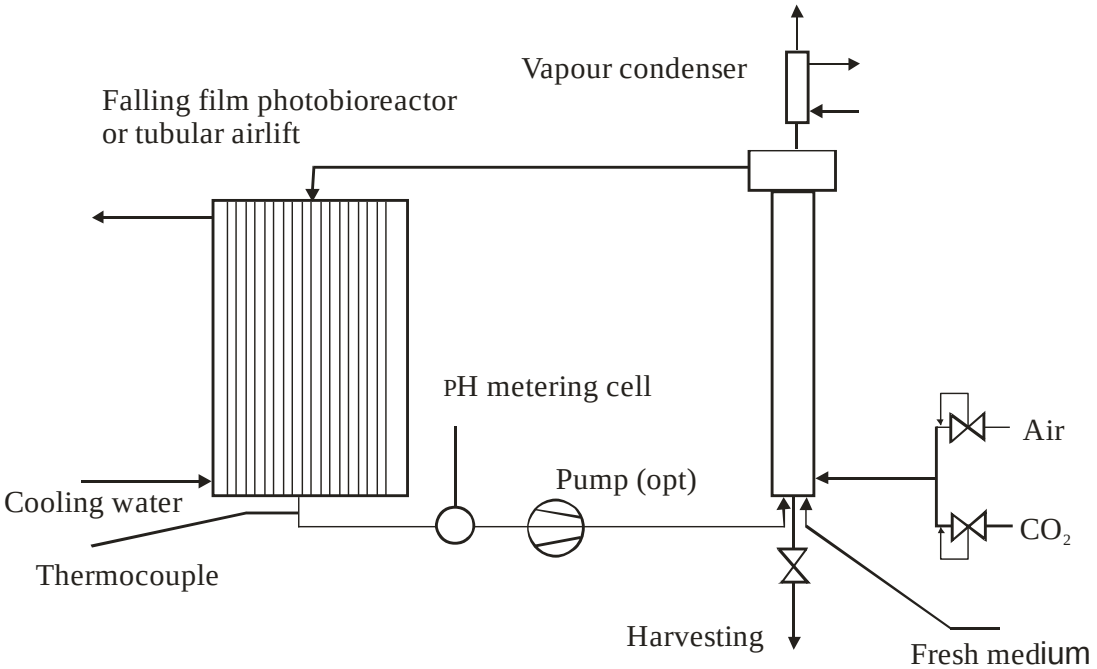


Figure 2:1 – Initial photobioreactor design: including tubular system with a falling film illuminated section

2.2 Overview of photobioreactor

The overall design of the photobioreactor was designed for optimal yield of phototrophic algae and flexibility of parameters (Figure 2:2). These parameters include varying light conditions, carbon dioxide concentrations and the temperature of the photobioreactor.

2.3 Construction materials

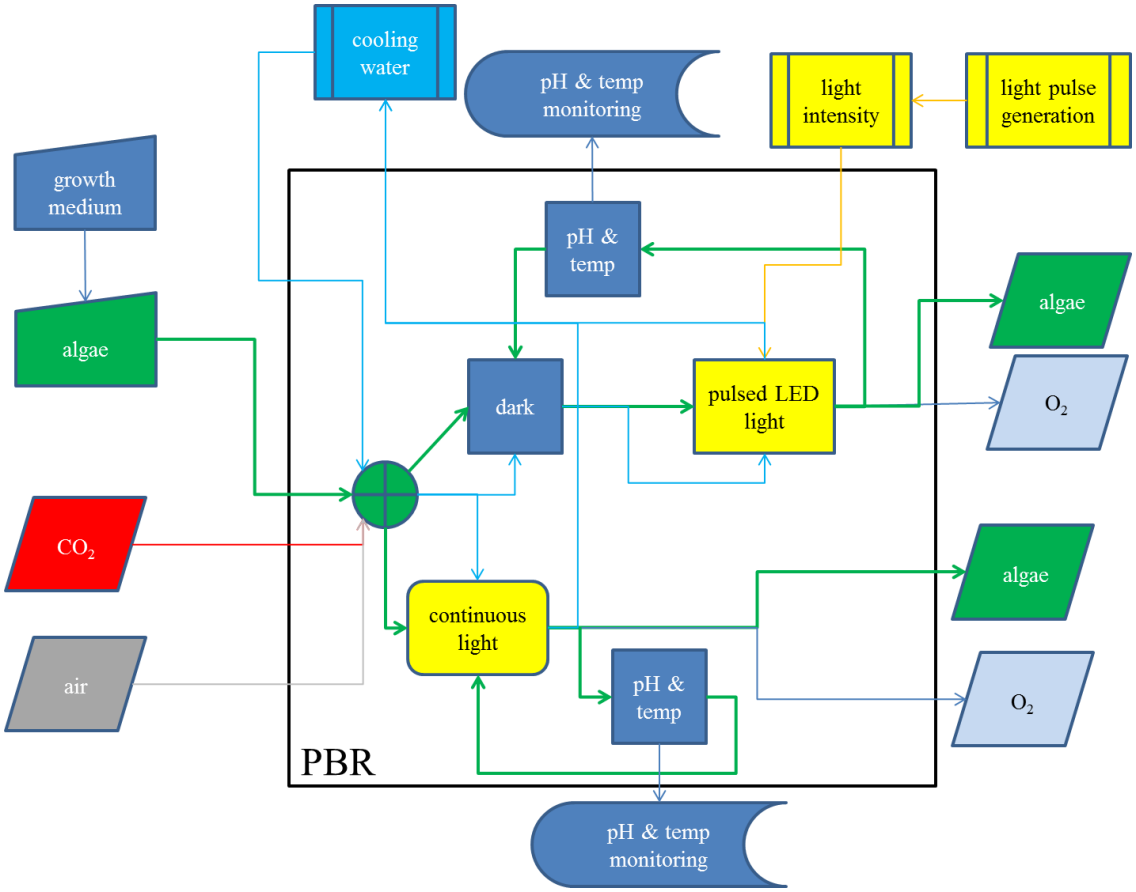


Figure 2:2 – Schematic of algal photobioreactor designed and constructed

All equipment and construction materials were researched and sourced using recommendations from technical staff at the *University of Bath* alongside systematic market searches using the internet and contacting of engineering representatives of several companies. Where newer materials, *e.g.* glass filled PTFE, have been used certain risks were inherent due to limited application of the materials and as such specifications of these materials were restrained. Glass filled PTFE was chosen due to its inert properties and it was expected to be easy to process, using similar techniques to those used for poly-ether-ether-ketone (PEEK) manipulation. PEEK was the preferred material for the intricate end connecting sections of the illuminated section, due to the experience of the technician, however it was unable to be used as it was not available in the required large blocks.

Glass filled PTFE is polytetrafluoroethylene filled with 15 % glass fibres and 5 % molybdenum disulphide powder, which has a density between 2.1 – 2.2 g cm^{-3}. Glass filled PTFE has a melting point of around 320 ^{o}C (ISO 54765), ignition temperature of more than 500 ^{o}C (ASTM D 1929), a decomposition temperature of >260 ^{o}C and a minimum service temperature of -200 ^{o}C; making it suitable for the application.[2] The properties of glass filled PFTE also allowed for the initial expansion of the glass to be taken into consideration throughout the usage of the photobioreactor due to its malleability.[2]

Some of the equipment and materials used were designed for specific applications which are different to these requirements, nevertheless the materials and equipment were chosen for their potential flexibility of use. Materials were chosen largely due to their mechanical and physical properties, whilst taking into consideration their potential impact on algae growth.

Table 2:1 – Negative exposure of S-starved cultures of *Chlamydomonas euryale* to different metal alloys, plastic and rubber[3]

Material	**Item (Company)**	**Old/New**	**Toxic effect**
Natural rubber	Red rubber stopper (Deutsche-Neumann)	New	+++++
Natural rubber	Grey rubber stopper (Saint-Gobain)	Old	(++)
Silicon rubber	Yellow silicon stopper (Saint-Gobain)	New	++++
Silicon rubber	GR-2 septa (Supelco)	New	-
Latex rubber	Latex glove (Ansell)	New	-
Polypropylene (PP)	Screw cap (Wheaton)	New	+++
Poly ethylene terephthalate (PET)	Screw cap (Schott)	New	(++)
Nitrile	O-ring (Kendro)	Old	(++)
Polystyrene (PS)	Sample vial (Elkay)	New	-
Polyethylene (PE)	Cap for sample vial (Elkay)	New	-
Stainless steel 304	Hypodermic needle (Becton-Dickinson)	New	++
Stainless steel 316	Gas connection (Supelco)	New	(++)

n+ : cell death n days before control
(n+) : colour change detected, but no increase in death rate detected
- : no effect
NB "old" items used and washed repeatedly (years)

Certain changes of materials, equipment and layout occurred throughout design and construction for unforeseen technical reasons. Experience gained early on in the design and build of this photobioreactor highlighted key issues which were given special consideration, such as the rigidity and misalignment of the main airlift section.

Algae growth can be affected by a variety of common construction materials (Table 2:1). Studies[3-4] reported in literature where specific materials are tested for their impact on algae growth have been analysed such that optimum growing conditions were aimed to be provided for algae in this photobioreactor. Silicon rubber, latex and natural rubber (made up of isoprene units with impurities including proteins, fatty acids and inorganic substances) have all been reported to have a negative impact on the growth rate of algae.[3] However silicon rubber septa, which were tested alongside the natural rubber and silicon, were found to have no impact on growth of algae. It has been reported that when older rubber is used there was less of an impact on algae growth than when new rubber was used. This implies that there was an initial leaching of detrimental compounds, but once this occurred the rubber or silicon was less toxic.[3]

Polytetrafluoroethylene (PTFE) has not been reported to have adverse effects on algae growth and is known to be relatively inert, so was utilised in the reactor design. Polystyrene and polyethylene have been reported to have no effect on the growth of algae, so these could have been incorporated into photobioreactor if required (although polyethylene is not resistant to autoclaving, so contamination could have occurred). Avoiding materials which hinder algae growth is important in the successful construction of reactors for algae, particularly if stress conditions and other parameters are being simulated and varied. Epoxy fittings were included in the reactor design, and have been used within algae reactors in literature resulting in no reported impact on algal growth.[3]

Certain metals are essential micronutrients for algae growth (see section 1.3.2); once the concentration of those metals is above the optimum level they quickly become toxic. The use of stainless steel and cast iron for construction of photobioreactor parts is problematic as they contain compounds which detrimentally affect the growth rate of algae (Table 2:1). Metals which are known to detrimentally affect algae growth in excess are chromium, nickel, molybdenum, mercury, copper, aluminium and lead.[5] Algae sometimes have the ability to adapt to changes in nutrient levels to provide interesting results. Some algae

species can detoxify aluminium and other metals by the formation of polyphosphate bodies. It has also been reported that lead and aluminium have a stimulatory effect to algae growth at low concentration and a detrimental/toxic effect at high concentration. There are changes to the biological ultrastructure of the algae when a toxic effect is observed.[5] It has been possible to exclude metal contact in the photobioreactor to the algae or growth medium, which ensured no impact on algal growth rate by an excess of metals.

Nitrile (used in O rings), tygon and polymers such as polyetherimide and polypropylene as well as biodegradable polymers have also been reported to adversely affect growth rate.[3-4]

Glass or translucent plastic is often used extensively for the construction of photobioreactors. Glass interferes less with incoming light radiation and is more durable than plastic. Borosilicate glass has a very low coefficient of expansion (3.3×10^{-6} / °C) making it more resistant to thermal shock and suitable for use at temperatures up to 500 °C.[6] Borosilicate glass has a superior durability, as well as chemical and heat resistance compared to regular glass. Borsilicate glass has a thermal conductivity value of 1.14 W m^{-1} K^{-1} which means it conducts heat faster than air and most plastics, but at a much slower rate than metals.[6] The borosilicate glass has a reasonably low refractive index across the visible range $(1.51 – 1.54)^{6}$, thus there will be less diffraction of light for photosynthesis. Glass was used significantly throughout the photobioreactor design mainly due to its minimal interference with light and inertness to algae.

2.4 Airlift section

The airlift section of the photobioreactor was selected and designed so that the different components of the reactor could be easily attached and integrated to the reactor. It ensured gentle mixing of the biomass culture without shearing the algae cells. The algal biomass was mixed and moved by the flow of air and carbon dioxide throughout the system, and set up of the pipes was optimal for the flow of algae biomass to enter the illuminated section to enhance the light penetration.

The airlift section was produced from glass and makes up a major section of the reactor. Borosilicate glass with PTFE (polytetrafluoroethylene) gaskets was chosen as a construction material as there are no negative interactions between glass and algae growth.

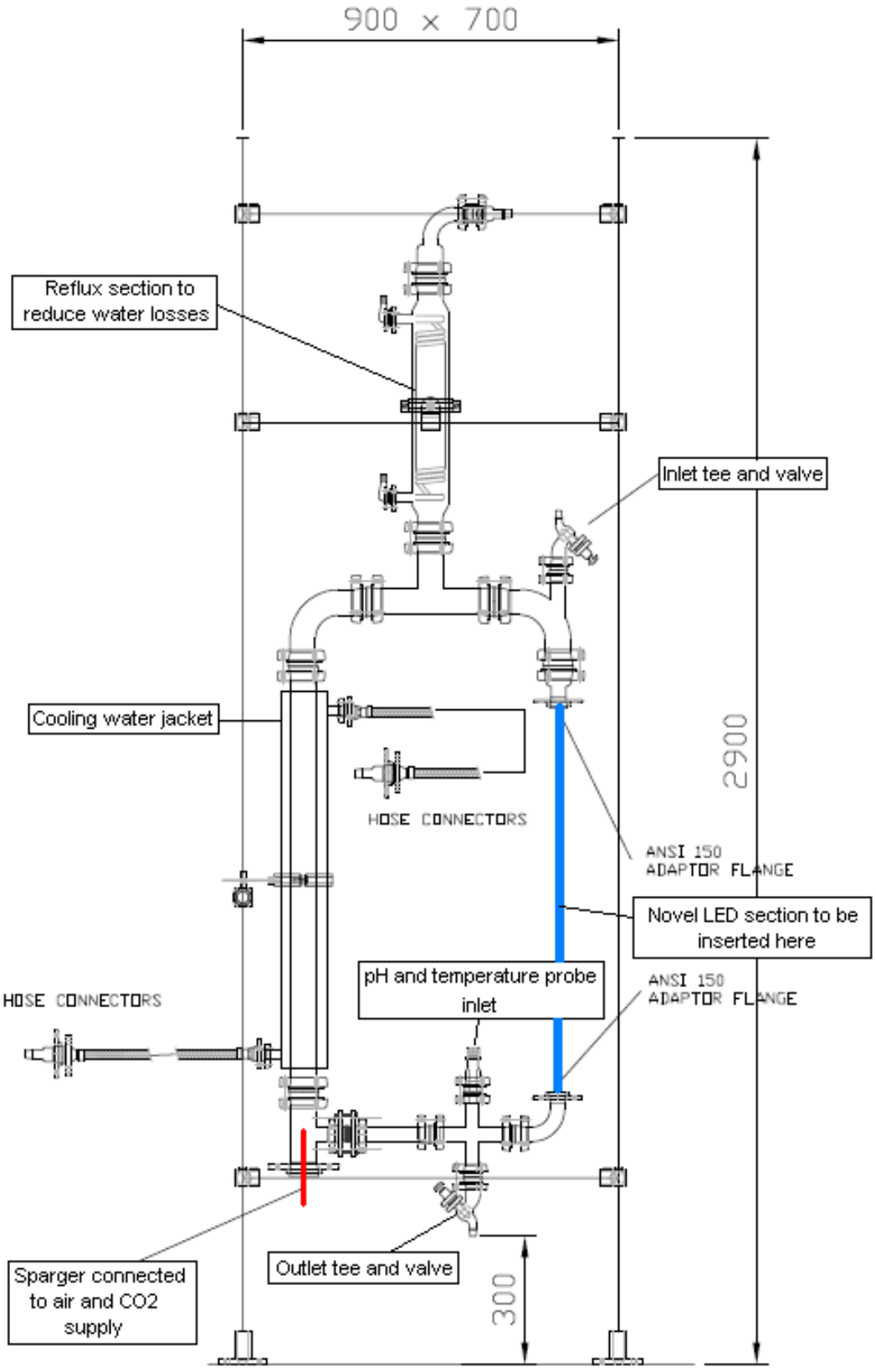


Figure 2:3 – Initial design of the borosilicate glass airlift section with PTFE O ring seals as designed and supplied by *QVF*, now part of *De Dietrich Process Systems* (all measurements in mm): the blue line is where the novel illuminated section was placed and the red line where the sparger was inserted into the system

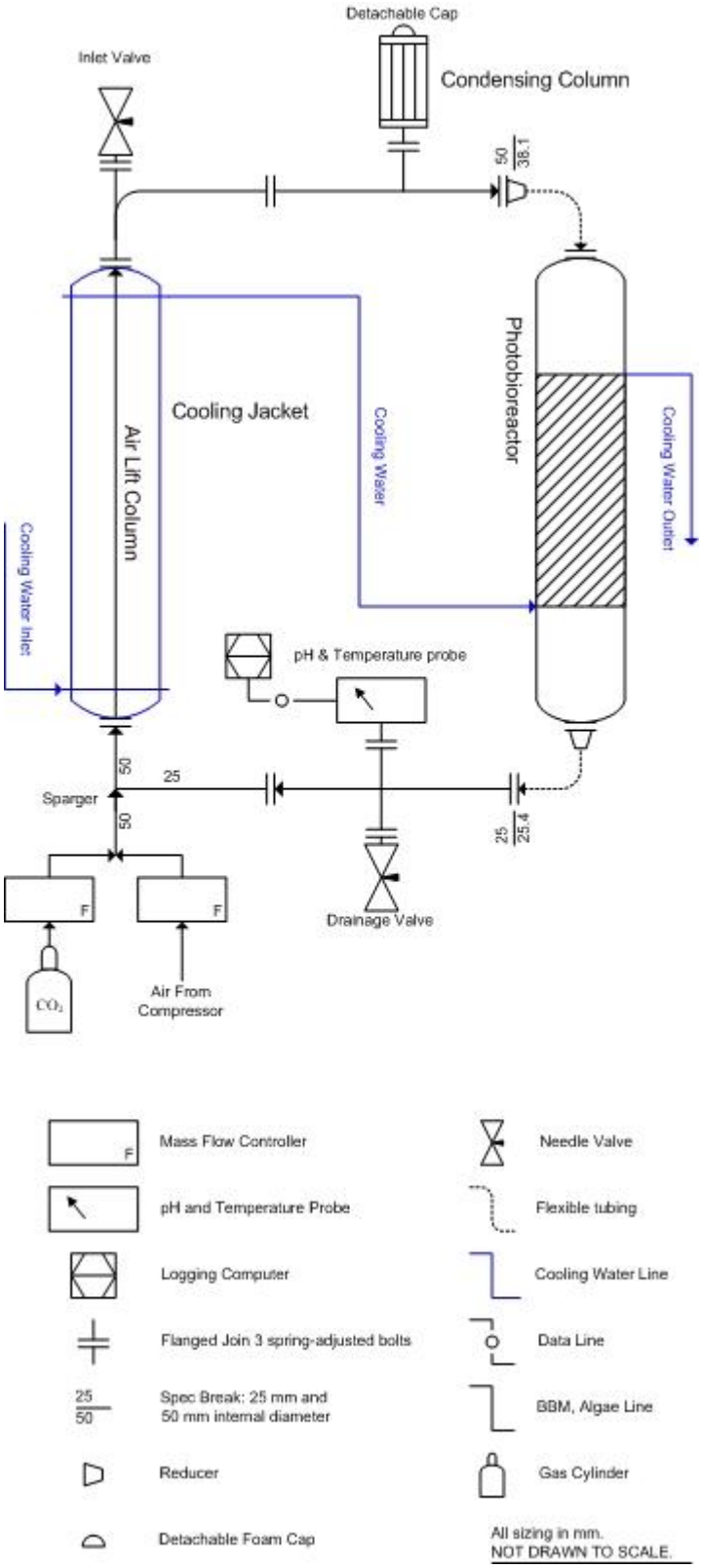


Figure 2:4 – Piping & instrumentation diagram of final photobioreactor design: with resultant location of components

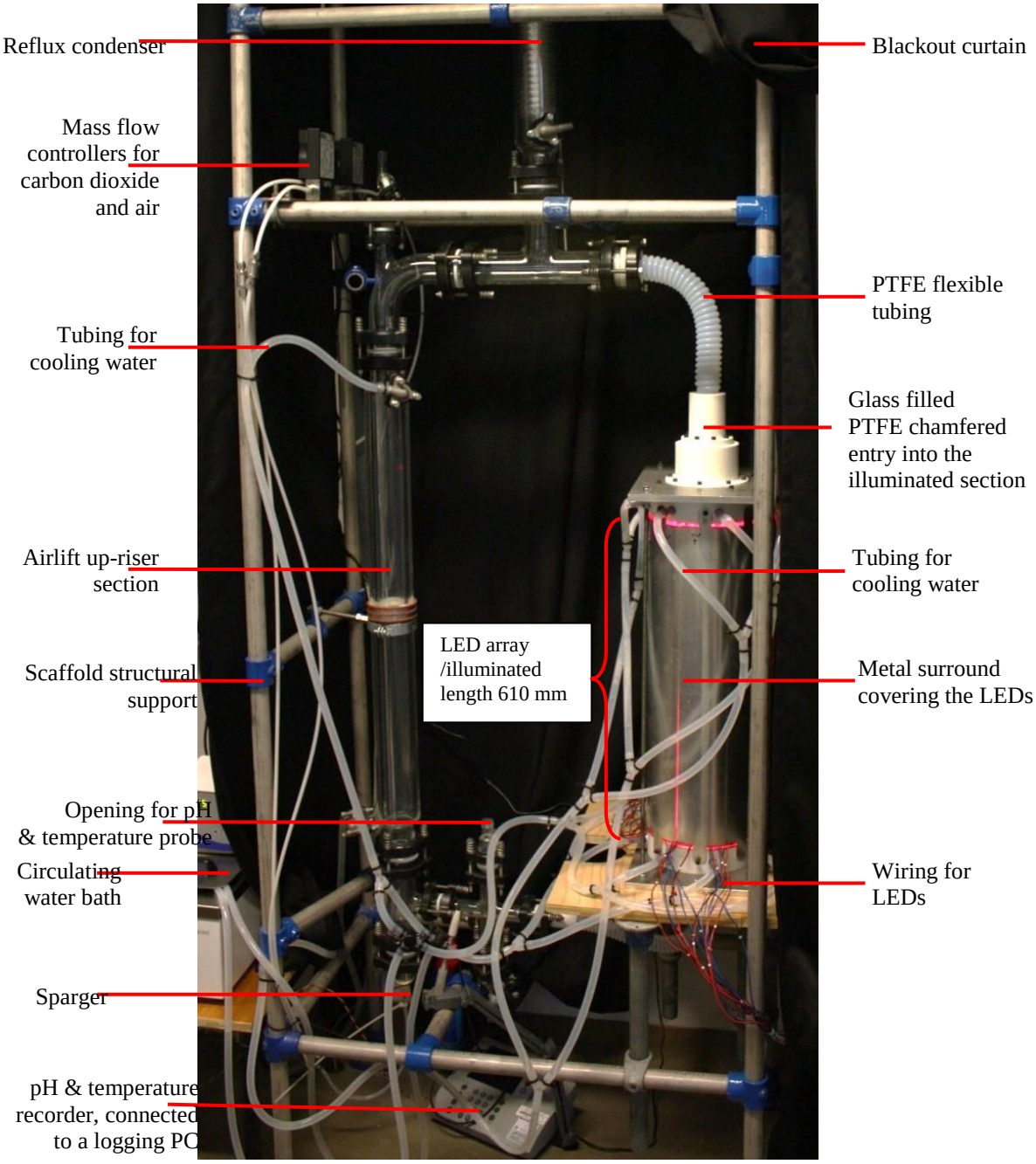
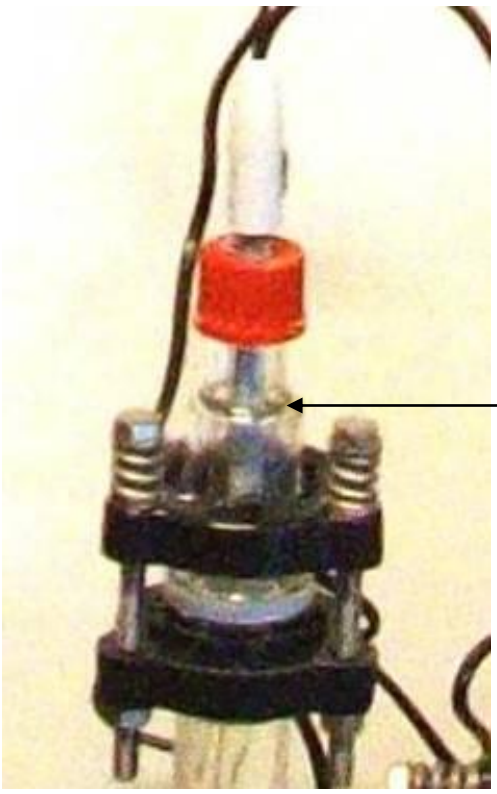


Figure 2:5 – Photobioreactor *in situ*, 2900 mm high, 900 mm wide and 700 mm deep, piping diameters of 25 mm and 50 mm

Agglomeration of the algae was easy to notice and clean, as were bacterial or chemical growths due to transparent properties of the glass. The airlift section was designed and produced by *QVF Engineering GmbH*, and provided by *QVF Process Systems Ltd*, Stafford, England (Figure 2:3). These pipelines have been widely used in the pharmaceutical, food and drinks, chemical and electroplating industries thus having been exposed to many different environments and uses, particularly where sterility is essential.

Figure 2:3 shows there were taps where nutrients and algae starting biomass could be admitted (top right hand side) and at the bottom where algae biomass and waste products could be removed and harvested. There was an entry point for the temperature and pH meter to be situated – though this did require some alteration, as it was an enclosed holder. The end cap section of this was removed so that the meter was immersed into the algae biomass to enable pH and temperature measurements to be recorded whilst remaining water-tight (Figure 2:6). The preliminary temporary addition of rigid nylon braided PVC tubing, in blue in Figure 2:3, allowed for testing of the supplied reactor, and the addition of a sparger, depicted in red to provide airflow for adequate airlift.



This fitting was sealed at this indent. It was cut to allow entry of the pH and temperature meter. A screw cap glass fitting was attached by the glass blower to allow a water-tight attachment.

Figure 2:6 – pH and temperature probe attachment and entry point

There was a cooling section on the left hand side which was connected to a water bath to maintain constant temperature for the algae biomass. The cooling jacket was also connected to the light reactor section cooling system so the same water and pump was used to remove heat from the algae growth medium in both sections (Figure 2:2 and Figure 2:4).

This glass airlift section was designed to be supported on a scaffold frame which was stabilised by attaching to the floor and wall. This gives the whole reactor maximum structural stability as the illuminated photobioreactor section was also attached to this modified scaffold support using a wooden platform; which ensured that no breakages

occurred. As can be observed from the progression from Figure 2:3 to Figure 2:4 and Figure 2:5 the positions of some of the components supplied by *QVF Process Systems Ltd* were altered to allow for attachment of the illuminated section without leaking. For example, the inlet tee and valve were moved symmetrically to the left hand side of the rig, and a DN25 90^{o} bend, a DN50 90^{o} bend, a DN25 glass to glass bellow and a DN25 by 175 mm pipe section were all removed. (The labelling of parts "DN50", for example, means pipe of 50 mm in internal diameter) This allowed for insertion of flexible PTFE tubing between the QVF parts and the illuminated section which allowed for adjustments to eradicate all leaking. As visible in Figure 2:5 a black out curtain was required to omit any interference to growth conditions from natural light or artificial light in the laboratory. The black-out curtain was tailored from fire retardant, black out material obtained from a local blind company. Measurements were taken of the scaffold rig and specifications drawn up; a simple dual rectangular cover was produced providing complete darkness. The curtains were removed for initial natural light experiments, but not for subsequent white light experiments.

2.4.1 Sparger

The sparger (Figure 2:7) was used to ensure that the carbon dioxide and the air entering the photobioreactor had maximum possible contact with the algae biomass to optimise mass transfer of the gas to algal cells. This was realised by having many small bubbles, an increase in surface area, which lead to a higher mass transfer rate. With the gas entering the photobioreactor through holes of 16 – 40 μm in diameter the flow of the algae biomass mixture was sufficient to ensure mixing and an airlift effect, whilst being gentle enough to not cause problems with shear forces and stress breaking or damaging the algae. It has been noted that the bubble size is independent of initial size at formation; it is controlled by the equilibrium of dynamic pressure and surface tension forces.[7] The bubble size released into the reactor is approximately 2 – 5 mm.

The sparger was inserted so that the top was above the bottom of the longitudinal to ensure airlift occurs homogeneously (as highlighted in Figure 2:7). Spargers can be produced from stainless steel, ceramic, glass and PTFE. The sparger was sourced from *DasGip AG*, Germany and was produced from borosilicate glass with pipe section 15 mm long and porous glass frit of pore size: 16 – 40 μm diameter. The glass and PTFE used ensured that there were no adverse effects on algal growth from the sparger fitting. The diameter of the

pipe section and the sparger end were 6 mm. The sparger was fitted to an end closure component as described in Figure 2:7.

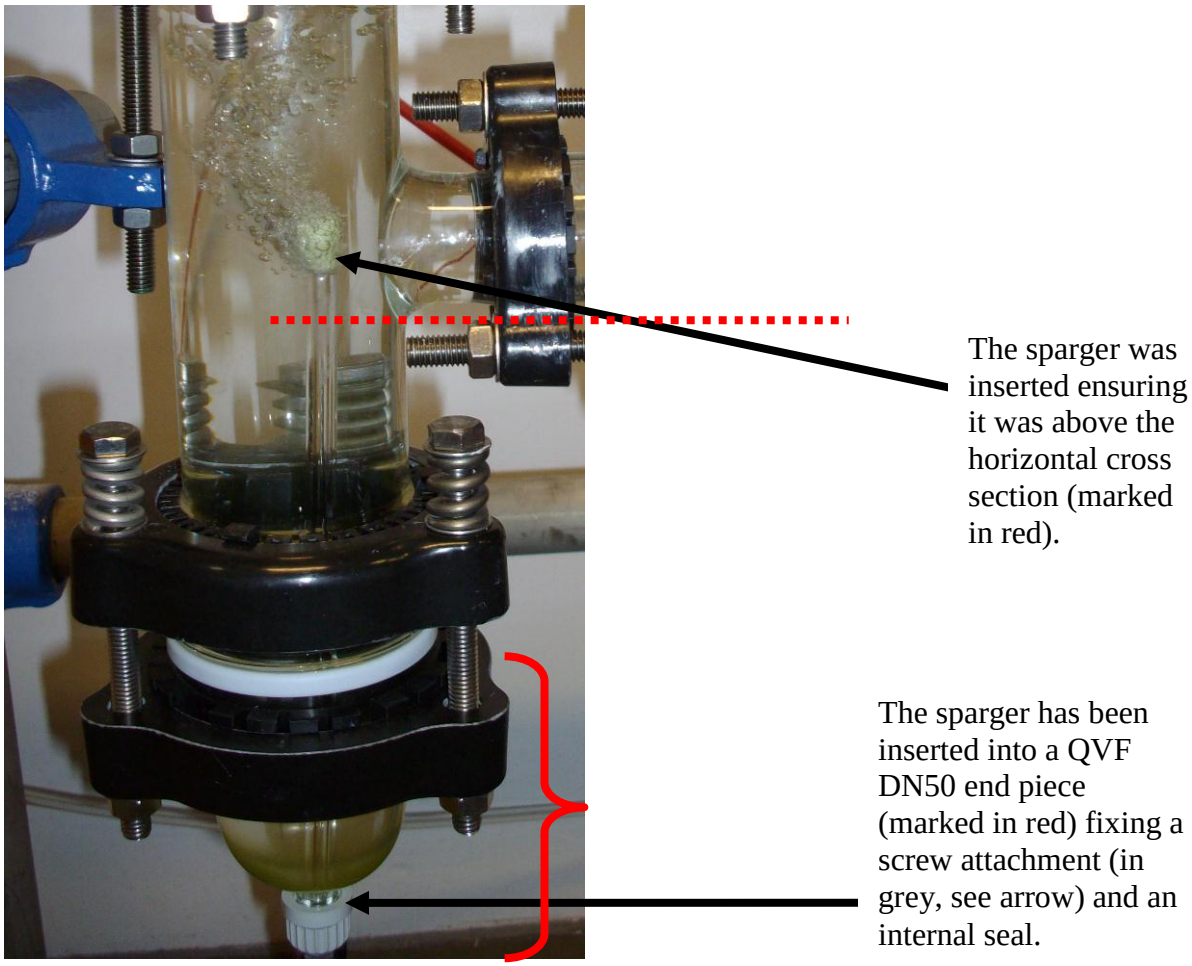


Figure 2:7 – Sparger attachment and entry

2.5 Illuminated section

The illuminated section (Figure 2:11 and Figure 2:12) was designed to optimise light input and path length as well as to optimise algae flow through the section. Light capture, usage and distribution are all critical for optimisation of algae growth. Therefore shading from other algae cells was kept to a minimum by the small width (5 mm) of the algae path through the light reactor section. This allowed for optimal penetration of light from the LEDs with regards to path length and diffraction within the algae biomass. It was hypothesised that only 30 – 40 % of the emitted light from the LEDs will reach the algae as the rest was predicted to be absorbed by the glass pipes and coolant water. The number of LEDs and their distance from the algae was chosen to provide uniform illumination to the

algae cells whilst obtaining optimal flux of photons for photosynthetic system saturation when all LEDs are illuminated.

Glass cylinders and glass filled PTFE were used due to their inertness to algae and chemical species; see section 2.3 regarding construction materials.

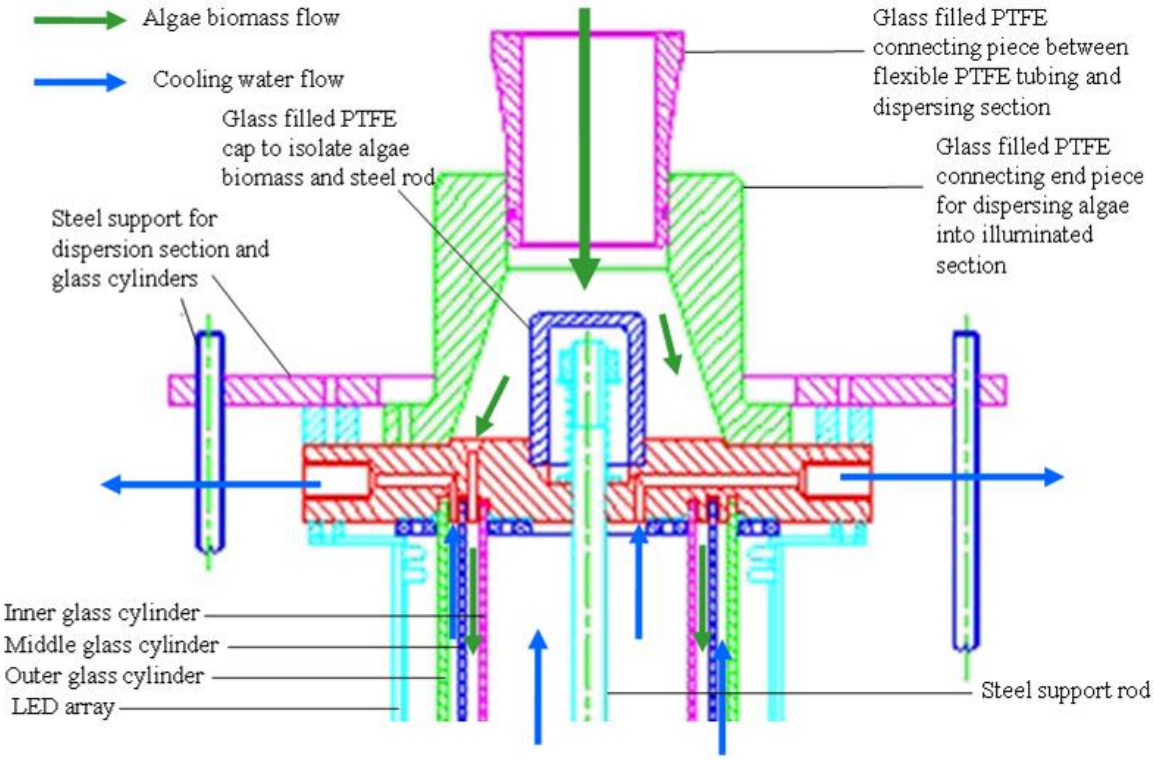


Figure 2:8 – Chamfered entry of algae into illumination section: the flow of the algal biomass through the chamfered entry (in pink) is depicted by the green arrows it flows downwards past the PTFE cap (in blue) which is sealing off the steel support rod (in turquoise) and through 20 3.5 mm holes (in the red section) into the illuminated area; the cooling water flows in the opposite direction, upwards, through the central cylinder (in purple) and the outer gap (between the blue and green cylinders) then out to the cooling water bath through eight channels (in red; four for the central channel and 4 for the outer channel)

As shown in Figure 2:8, Figure 2:9 and Figure 2:11 there was a supporting steel rod to ensure the glass cylinders were not crushed by the weight of the top end manifold or the tension when the end manifolds were sealed to the cylinders. This steel rod was only in contact with the internal cooling water and had a glass filled PTFE cap at both ends; where it protrudes into the end manifolds and would otherwise have been in contact with the algal biomass.

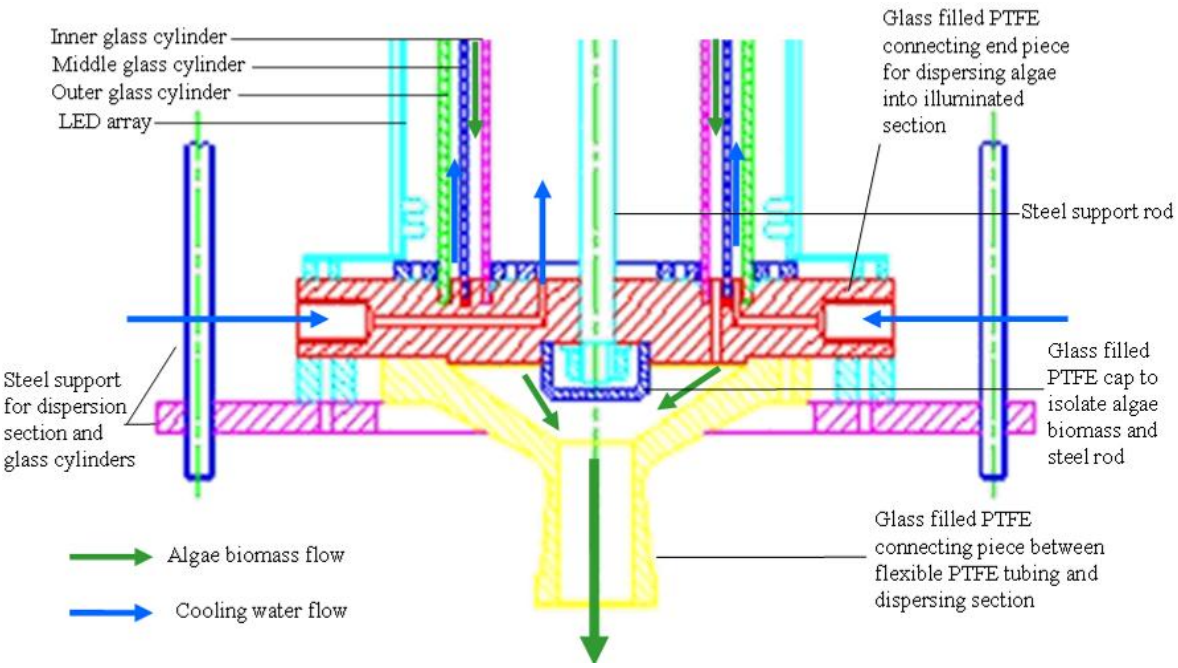


Figure 2:9 – Chamfered exit of algae from illuminated section: the flow of the algal biomass out through the chamfered entry (in yellow) is depicted by the green arrows it flows downwards through the illuminated section between the cylinders depicted in blue and purple and out through the 20 3.5 mm holes (in the red section) past the PTFE cap (in blue) which is sealing off the steel support rod (in turquoise); the cooling water flows in from the cooling water bath in the opposite direction, upwards, through eight channels (in red; four for the central channel and 4 for the outer channel) and into the central cylinder (in purple) and the outer gap (between the blue and green cylinders)

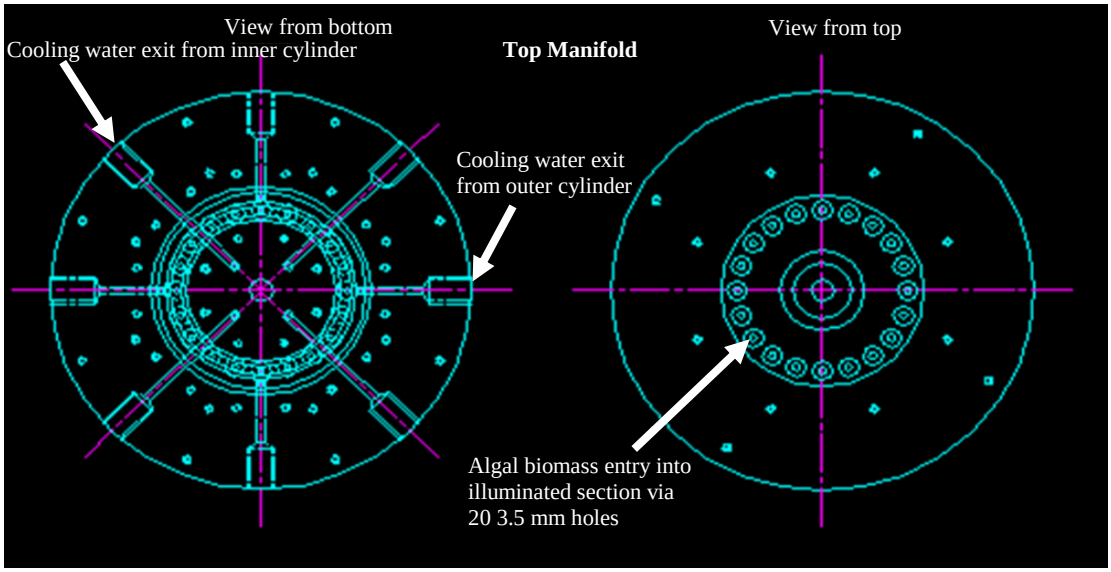


Figure 2:10 – Entry manifold for illuminated section, with entry holes for cooling water and for algal biomass highlighted: there were 20 3.5 mm holes for the algae biomass to flow through and 4 entry/exit points for the central cooling water isolated from the 4 for the outer cooling water

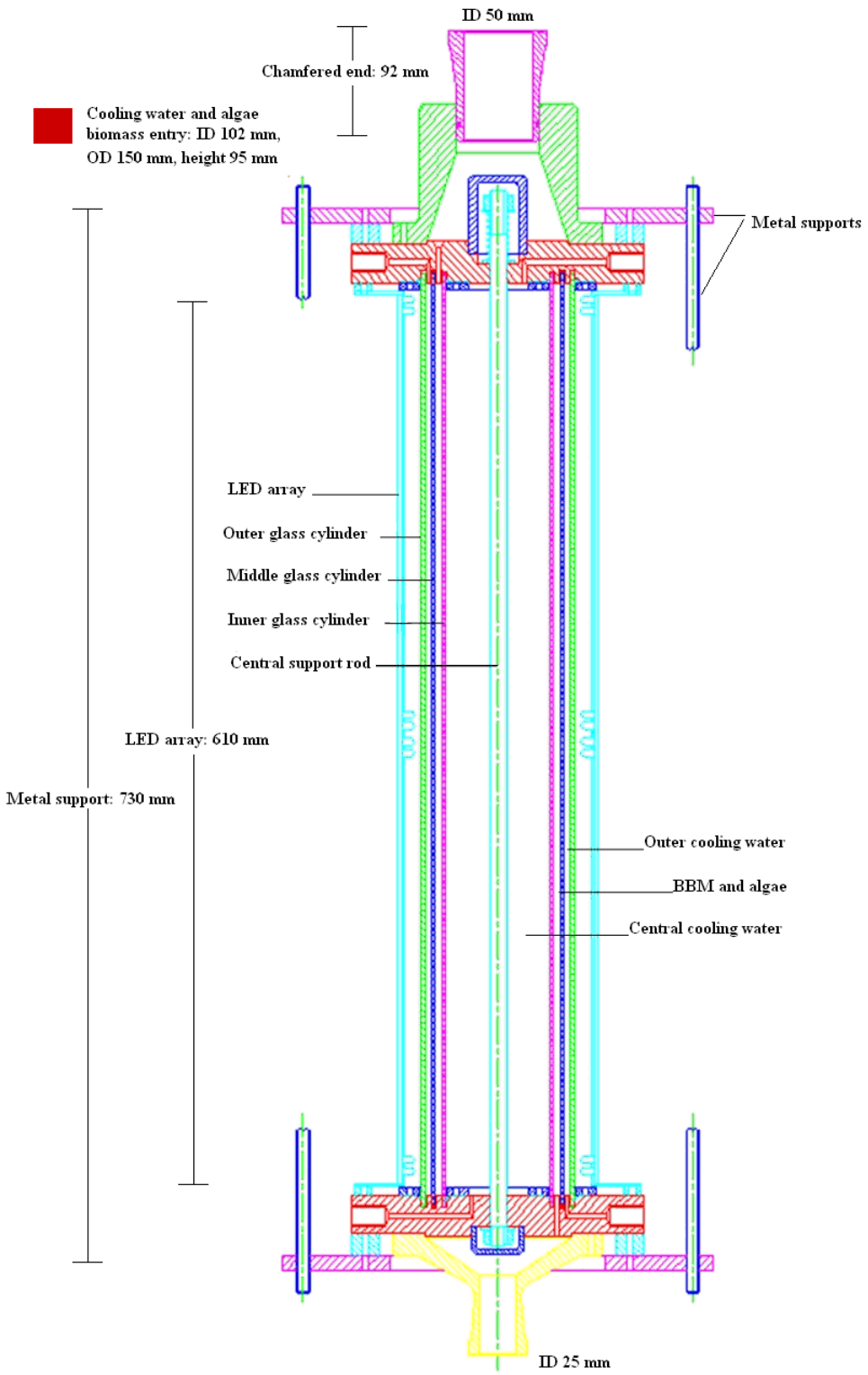


Figure 2:11 – Illuminated section of photobioreactor: illuminated length 610 mm

Steel in the form of steel plates was used to mount the two end manifolds to give rigidity and sustain the correct position of the different components of the illuminated section. There were four supporting steel rods which were tightened upon movement of the

illuminated section to protect against distortion of the illuminated section which would result in shattering of the glass cylinders.

The end manifolds where the algal biomass entered (Figure 2:8) and exited (Figure 2:9) the illuminated section was calculated to require a minimum of 10 holes with a diameter of 3.5 mm each to obtain a maximum flow rate of 10 L min^{-1}. To ensure that the flow rate through the illuminated section end manifolds was not a limiting or stress factor for the photobioreactor the number of entry and exit holes was increased to 20 of each (Figure 2:10). This increased the flexibility of the reactor to be used, in the future, for other experiments which may require an increased flow rate as well as ensuring laminar flow throughout the illuminated section (see section 2.5.3). The entry and exit holes were designed to be offset from one another to encourage laminar flow rather than direct flow from the entry hole to an exit hole exactly below. Due to the efficient chamfered shape and smooth surface finish of the entry and exit manifold from the illuminated section, there was an increase in flow rate compared to a more modular design.

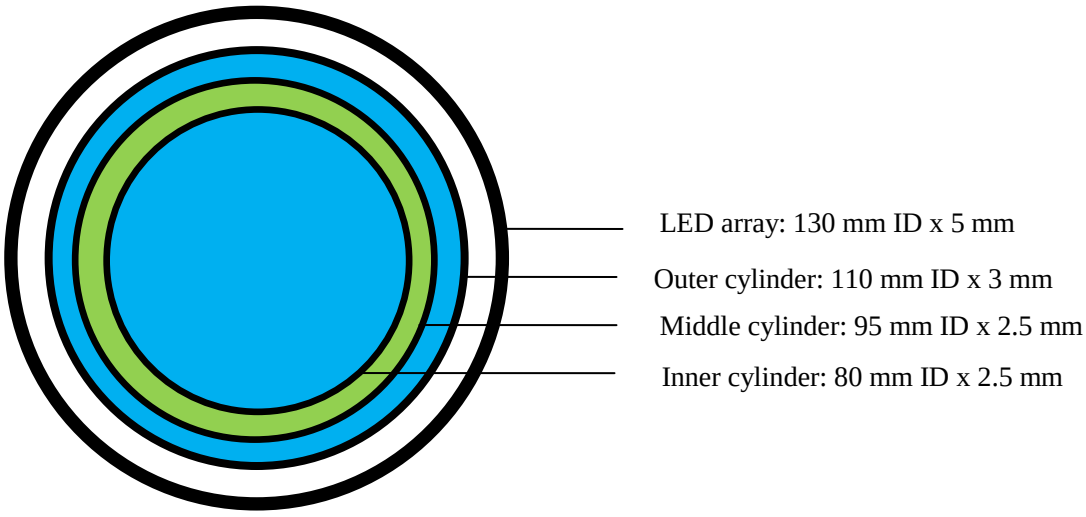


Figure 2:12 – Cross section of illuminated section of photobioreactor: the LED array has an internal diameter of 130 mm and a thickness of 5 mm, the outer borosilicate glass cylinder has an internal diameter of 110 mm and a thickness of 3 mm, the middle cylinder has an internal diameter of 95 mm and a thickness of 2.5 mm and the inner cylinder has an internal diameter of 80 mm and a thickness of 2.5 mm; the blue depicts the water channels and the green the algae biomass channel

2.5.1 Temperature and pH logging

The temperature and pH of the algae growth medium were monitored using *200 series model 250* sensor from *Denver Instruments*. The temperature was kept constant using the circulating water bath detailed below (2.5.2); cooling was required to achieve this due to heating resulting from the LED illumination.

A *LABview* programme for data acquisition (see appendix), was used to log and record the outputs of the electrode sensor. The programme, controlled by the block diagram shown in the appendix, ran continuously until the stop conditions were met; when the stop button was pressed on screen. The temperature and pH readings were logged, via a *HyperTerminal* connection, on the computer every 5 minutes using a *LABview 2010* programme. The use of logging and regular monitoring of the pH and temperature of the algal biomass ensured continuity in the growth conditions. Other parameters, such as dissolved oxygen, phosphate and nitrate levels, were able to be tested using *Merck Spectroquant* cell tests, all of which required periodic testing of sample aliquots via samples taken from the outlet valve. Growth rate was measured via manual and automated cell count as required.

2.5.2 Cooling

Cooling was achieved by a jacketed column with circulating water from the circulating water bath. This water bath was a *Fisherbrand 3016H heated standard controller 115 V 60 Hz (220-240 V) 6 L* with a working temperature range between ambient +15 °C to 150 °C. On the alternate side of the reactor the illuminated section had two sections of cooling water within the concentric glass tubes. The algal biomass flowed through the middle gap (highlighted in green, Figure 2:12) and the cooling water travelled through the outer and central gap (shown in blue, Figure 2:12), also see Figure 2:8 and Figure 2:9. It was initially expected that the outer cooling water would be more than sufficient, however with preliminary runs, it was soon realised that the inner cooling water would be required too. These two sections were entirely independent of one another, enabling flexibility of removing one of the concentric glass tubes if a larger volume of algae is desired. The design of cooling flexible tubing had taps in to allow for the isolation and flow control of either section of cooling. The water bath was able to maintain pumping of water continuously for over 3 months. The observation of constant temperature readings for a period of 3 month continuous operation provides sufficient evidence of temperature control

of between 21 – 23 ^{o}C which is optimal for most green algae, including *Chlorella* spp.. The cooling water was most efficient if the light passed through it before it got to the algal biomass and therefore the heat energy had opportunity to dissipate.

2.5.3 Light emitting diodes illumination

The LED set-up to provide the illumination for the photobioreactor was designed to optimise irradiance of the algae as well as the temporal and spectral output of light for increased photosynthesis. LEDs were chosen as the light source for this design due to their ability to pulse with discrete and specified light and dark intervals. The LEDs were soldered onto 24 printed circuit board strips (Figure 2:13); these strips were fitted around the outside section of the illuminated section on a metal mount attached to the bottom manifold. The illuminated section had the flexibility to add supplementary LEDs into the centre of the section if necessary, by isolating the central cooling water section inside the inner 80 mm cylinder (Figure 2:12). There was also the opportunity to increase the intensity of the LEDs by removing the outer 110 mm cylinder (Figure 2:11) and placing the strips closer to the algal biomass. Neither of these alternatives were tested.

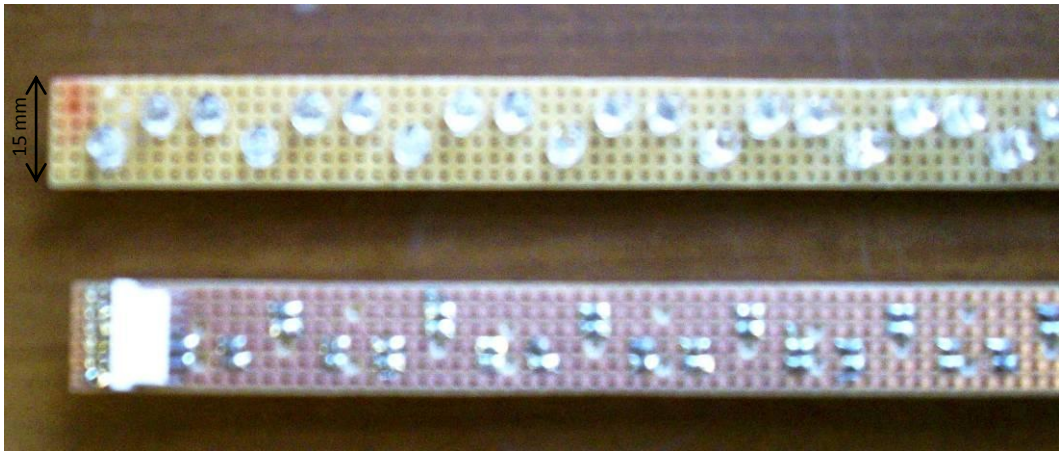


Figure 2:13 – LED strips front (top); the top row are red and the bottom row are blue LEDs (see Figure 2:14); and the back of the LEDs to show the connections (bottom), the white plastic section is where the electrical connectivity is added to the LEDs; the red LEDs and the blue LEDs are connected and controlled separately and are connected in series

The LEDs used for the photobioreactor had the highest luminosity ranking from *www.dotlight.de* and were manufactured by *Kingbright*, Ultrabright deep-red, peak wavelength 660 nm made from GaAlAs , LED 5 mm, intensity type: 4000 mcd, viewing angle: 30 °, with a forward voltage of 1.85 V and a current of 30 mA and are operational between -40 and +85 ^{o}C. Blue LEDs were also purchased from the same company which

had an intensity type of 12000 mcd and a viewing angle of 18 ^{o}, a peak wavelength of 470 nm and a current of 20 mA, forward voltage 3.6 V, operating temperature between -40 and 80 ^{o}C and produced from GaN/SiC.

Surface mount assembly (SMA) connections were used alongside printed circuit board (PCB) strips which were used for mounting the LEDs (light emitting diodes) in series (Figure 2:13).

The LED illuminated section consisted of 24 strips of 603 mm with a 15 mm width each (Figure 2:13). The thickness of each strip was around 1 – 2 mm plus the LED – which is about 10 mm in length. Each strip contained 52 red LEDs and 26 blue LEDs corresponding to the absorbance of chlorophyll of these regions of light (see section 1.5.10). The LEDs were arranged on each strip in an alternating formation of 2 red LEDs on the left hand side of the strip and 1 blue on the right (Figure 2:14). Each strip of LEDs also required a resistor; LEDs were not connected in parallel as this would have required an increase in the number of resistors used. The resistors were contained within the switching boxes and restrict the current to 20 mA per strip. A high voltage, around 120 V, was required due to the series connection of LEDs as each LED requires 1.9 V for red LEDs and 3.6 V for blue LEDs.

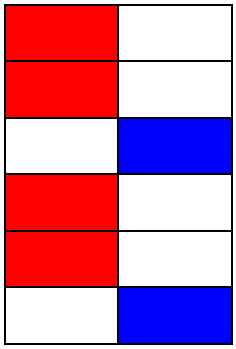


Figure 2:14 – Schematic of LED arrangement

Each of the LED strips was connected to two switching boxes, one for the red LEDs and one for the blue LEDs; these controlled the flow of electricity to the individual strips to allow for variance in intensity. Switching boxes were designed and produced to provide high levels of control of light exposure of the algae; see Figure 2:16 and Figure 2:17. The series connection of the LEDs meant that if there was a fault with one of the LEDs the whole strip would have been affected, leading to the faulty strip requiring removal, testing and the LED at fault being replaced. Identification of faulty strips was possible due to the

connection of LEDs in front of the switch on the switching boxes (Figure 2:17), if this was not alight at maximum voltage, the strip was not illuminated and there was known to be a fault within the strip.

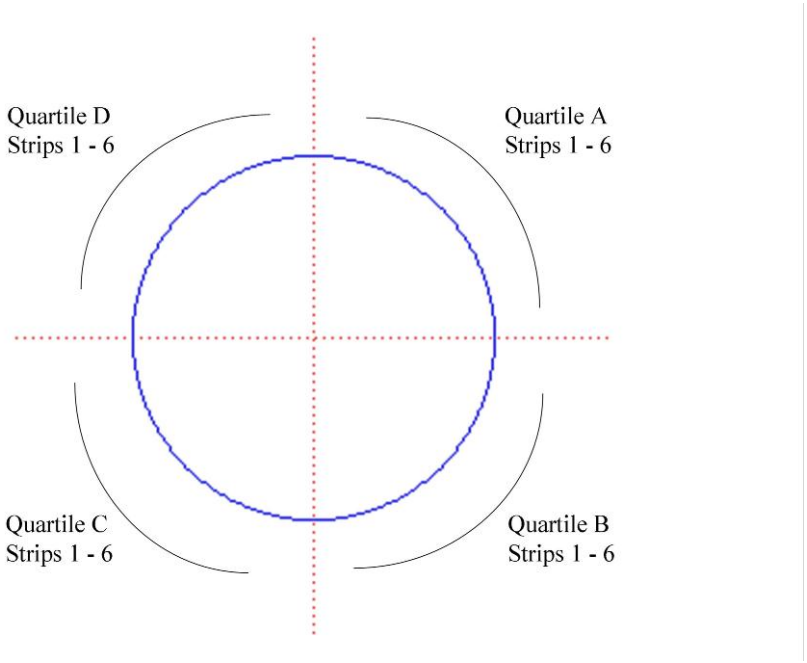


Figure 2:15 – Schematic of switching box control areas for LEDs

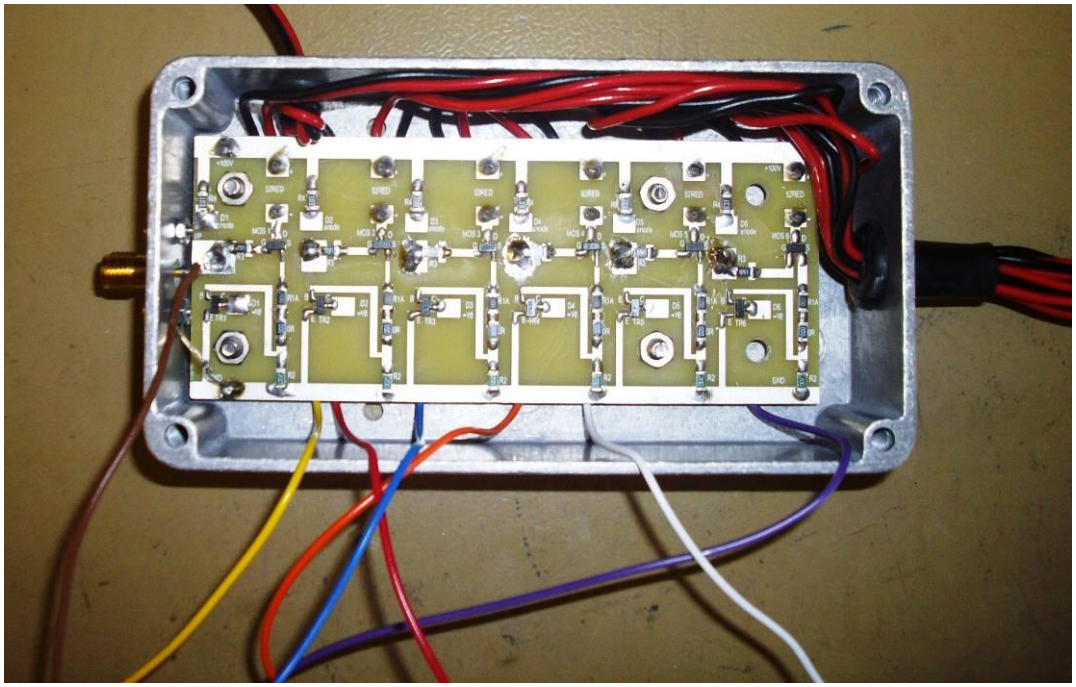


Figure 2:16 – Inside red switching box showing the circuit board with components attached: one box controls 6 strips of red LEDs

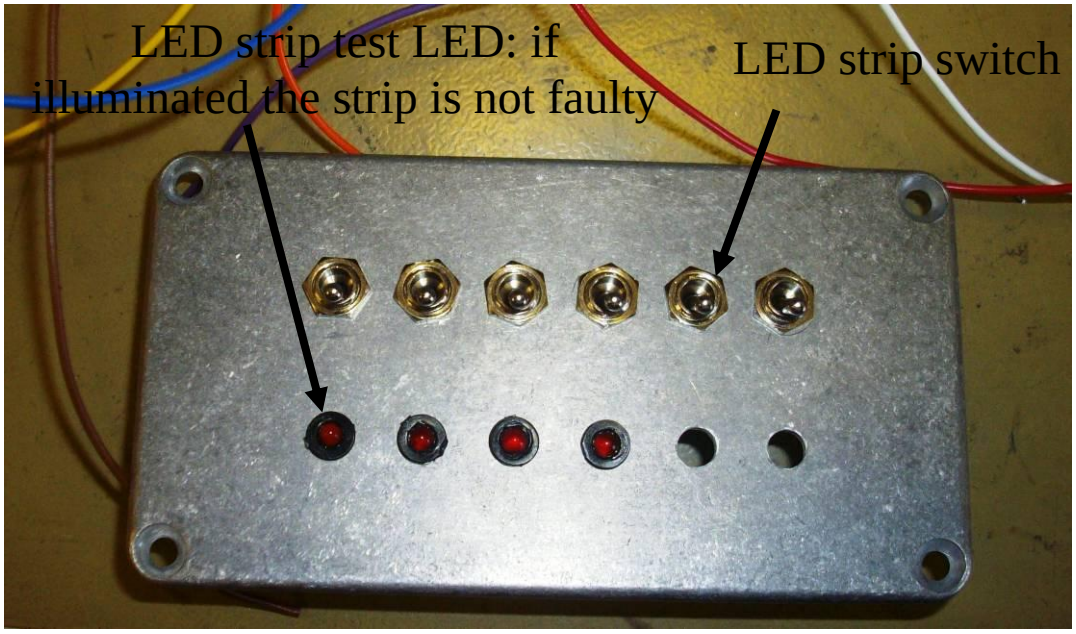


Figure 2:17 – Outside red switching box (under construction) with switches and LED test for each strip within the quartile

There were four switching boxes each for red and for blue LEDs; each of the boxes control six strips. These strips were placed together in order in a quartile of the LED mount (Figure 2:15) which allowed for homogenous distribution of light whilst allowing alteration of the intensity between 17 %, 33 %, 50 %, 67 %, 83 % and 100 % of capacity. There was also the flexibility to test solely blue light and solely red light on algae growth (see switching boxes Figure 2:16 and Figure 2:17). The switching boxes allowed for maximal control over the intensity and wavelength of light to which the algae are exposed.

In the switching boxes MOSFETs (metal (gate) – oxide (insulation) – semiconductor (silicon) field-effect transistor) were used to insulate between the each gate and body. The MOSFET controlled the flow of electrons, via size and shape of the conductive channel and the voltage, across the gate and source. MOSFETs provide adequate heat sinks within themselves to dissipate the large amount of heat generated and to avoid overheating; this is an advantage over using excess resistors.[8]

The pulsing of the LEDs was introduced by two pulse generators; this allowed for independent pulse widths and light times for red and blue light. Microsecond to nanosecond pulsing was attained using pulse generators (*Thurlby, Thandar TG 105: 5 Hz – 50 MHz*). One power supply unit (*TTi 300W Multi-mode Dual 75V/150V AC/DC Bench*

Power Supply) was used to supply sufficient power to all of the LEDs as the current was kept low, < 5 Amps, due to the series connection of the LEDs.

The LED part of the illuminated section with red switching boxes and 6 strips (red only) lit is shown in Figure 2:18. The box on the left of the figure was the switching box which controls the pulse lengths and dark periods. The power supply unit on the right provided the power in direct current format to the LEDs, controlled the voltage and displayed the current.

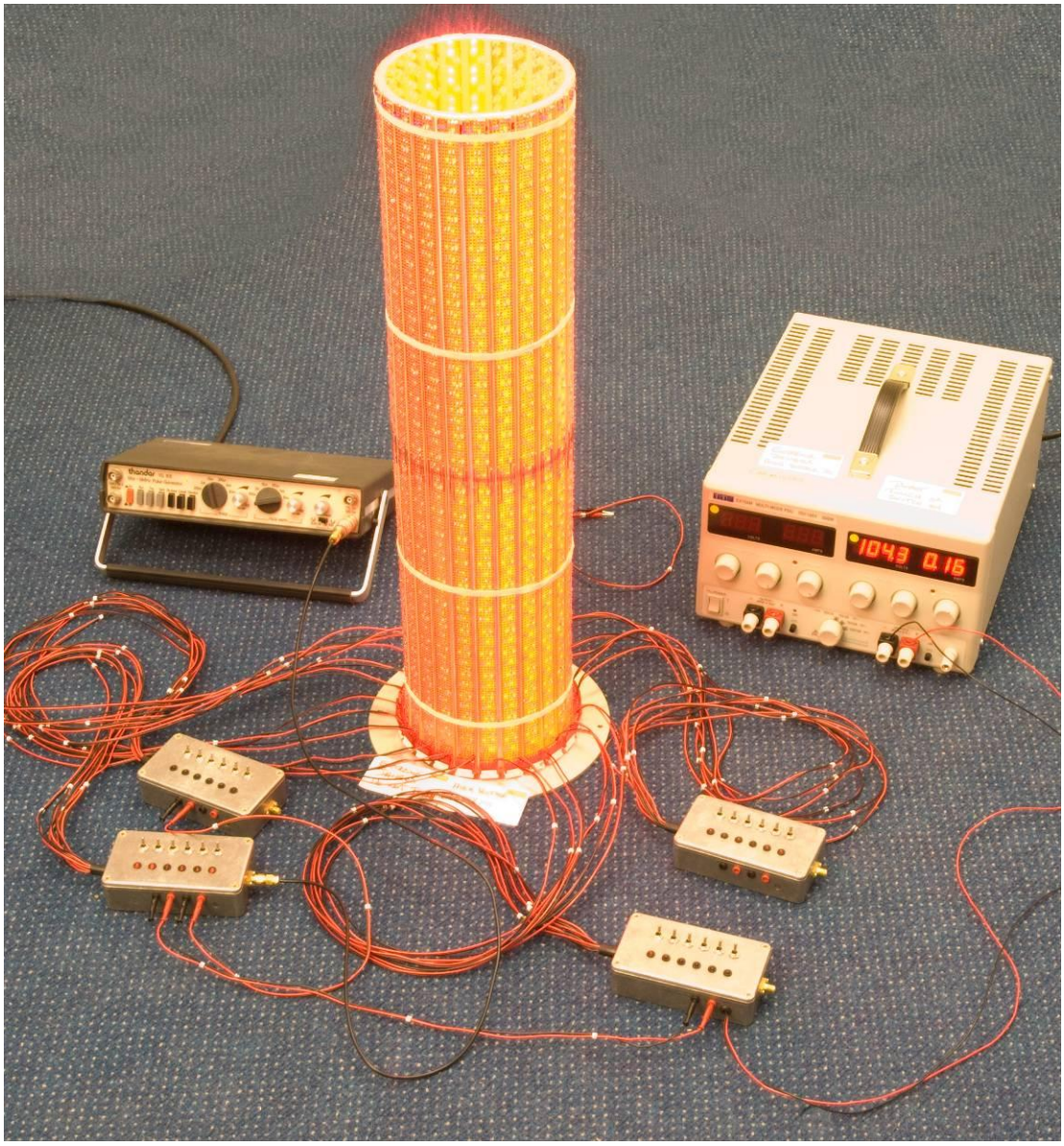


Figure 2:18 – Unattached LED section of the illuminated column of the photobioreactor – red LEDs solely illuminated (height of LED column – 610 mm) attached to a steel construct with 4 red switching boxes, one pulse generator – for the red LEDs (top left) and one power supply unit

The LEDs were fixed at a 7.5 mm distance from the outer glass cylinder, thus being between 17.5 and 22.5 mm from the algae. The intensity of the light from the LEDs reduced in a $1/r^2$ manner, at most, as this is the decay in energy with distance for a point source. The intensity reduction may be less than this as the LED range had a 10° radius. The available light intensity at a particular point within the illuminated section of the photobioreactor is resultant upon the incident light at the point of entry into the reactor, the concentration of biomass and the distance of the light path.

The glass cylinders used in the construction of the illuminated section are 629 mm in length, and due to their incorporation into the end manifolds the length exposed to light is 605 mm. This gave an algal lit area of 1284.3 mm^2 = 0.808 litres (approximately for a length of 605 mm) and flow calculations (of Reynolds number) show that the flow was laminar (that is RE <2400, above 3000 turbulent flow occurs), thus viscous forces are dominant. This means that there should be smooth, constant fluid movement with little impact of inertial forces and therefore no random eddies or vortices. Literature shows that as Reynolds number increases within the photobioreactor the growth rate of algae increases.[9]

Turbulent mixing is often used in algal growth systems, including open ponds and photobioreactors, to keep the algae in suspension and to ensure adequate illumination of all algal cells; however the growth enhancement this yields varies from algal growth system. As this photobioreactor was of airlift design the use of turbulent mixing would be detrimental to the overall flow of biomass. The use of turbulence within open ponds enhances depth and surface mixing to compensate for light penetration at the surface. It is also important to take into account other requirements of mixing, as well as for illumination purposes, the adequate supply of nutrients and carbon dioxide to cells and removal of oxygen.[9] The use of laminar flow ensured sufficient supply of nutrients and carbon dioxide, whilst allowing block flow of the algae throughout the airlift system. Laminar flow meant that the algal cells experienced flashing light on the time scale set by the pulse generator, and not dictated by the flow parameters.

Suitable precautions were taken to ensure the high voltage (around 120 V, direct current - unidirectional flow of electrical charge) was made safe. This included a polycarbonate case to contain all switching boxes and live wires has been constructed, as well as the LED

strips being surrounded by a steel protective shield. The power supply unit, pulse generators and switching boxes along with the associated wiring were fitted inside this customised polycarbonate box to minimise access to live equipment. Customisation of the box required a hole in the left hand side for wires to the illuminated section to be cut, a hole in the back for cables to plug sockets for the power supply unit and pulse generators, and eight holes in the top casing for the switching boxes to be attached for access to the control switches for individual red and blue strips.

2.6 Mass flow control and fluid dynamics

The mass flow control meters purchased from *Litremeter* (*100 range* – for air and for carbon dioxide) controlled the flow of the gas as well measuring the flow, within specific limits. Thus the inflow of gas rate had to be closely controlled to enable the required flow rate to be obtained and maintained. The *Sierra mass flow control meters* (Figure 2:19) were particularly sensitive to changes in incoming gas flow rate. The mass flow controllers were placed in series between the incoming gas source and the junction where the two gases mixed to enable exclusive control of each flow rate. Control of the flow of the gas was achieved by use of gas regulator for carbon dioxide and initially for the air supply too. After establishing that the growth conditions were not sensitive to the source of air, the centralised in-house compressed air supply was used to provide the air lift in the bubble column. The change of air supply was also amended as initially 120 L $hour^{-1}$ (2.00 sL min^{-1}) air was being used, which required the replacement of air cylinders every three days, this was neither economical nor practical. The pressure of the compressed air supply was sufficient for the mass flow control meter to maintain a steady flow of 2.00 sL min^{-1} whilst the carbon dioxide meter is set to 0.500 sL min^{-1}. Each mass flow control meter was calibrated for its specific gas by the manufacturer and calibrated to work with high accuracy for that particular gas.[10]

The flow through the mass flow control meters was found to not be consistently controlled by the mass flow control meters if the gas flow, of either carbon dioxide or air, was notably higher than the desired output. The control section of the meters was not working sufficiently well for experimental control over gas supply. Two pressure needle valves were consequently added upstream to the mass flow control meters which allowed consistent, specific gas pressure outputs to be maintained. Testing with an electronic flow

meter and a bubble flow meter corroborated the digital readings given by the mass flow control meters.

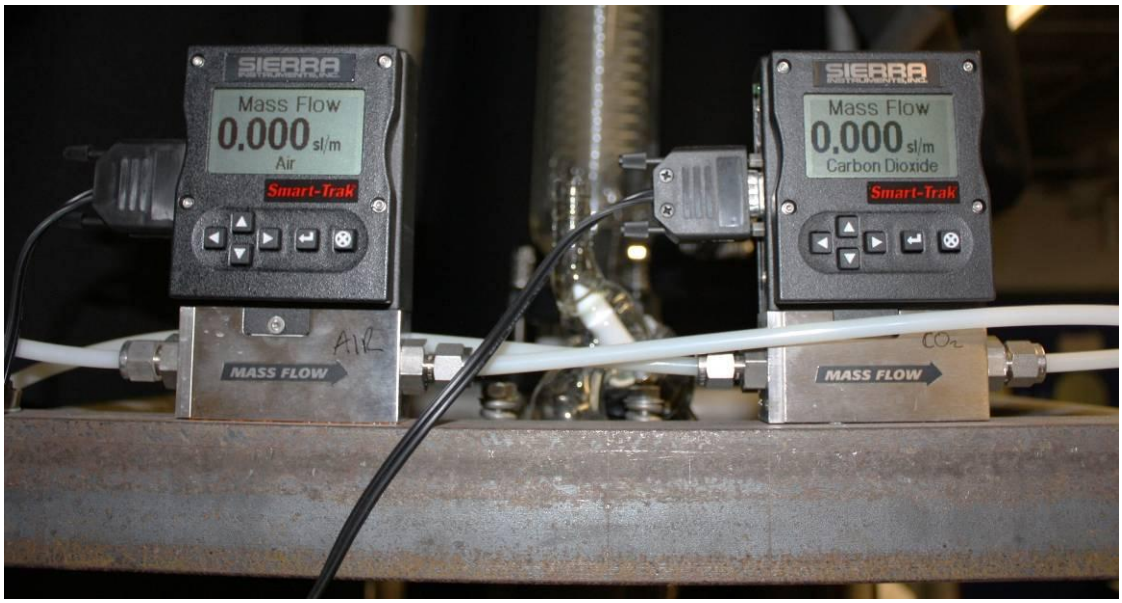


Figure 2:19 – Mass flow controllers: left hand side for air and right hand side for carbon dioxide – enabling the measurement and control of the flow of gas into the airlift photobioreactor

The flow rates for both carbon dioxide and air were reduced inline with the flow rates used in the Department of Biology, *University of Bath* where successful algal growth was initially obtained. The new flow rates of around 0.150 sL min^{-1} for carbon dioxide and around 0.400 sL min^{-1} for air were adequate for block flow to be maintained throughout the photobioreactor (see section 2.8.3).

The fluid dynamics of the system have been tested using coloured dye entered at the entry point and recorded the movements of this dye in water throughout the photobioreactor; block flow was achieved with no back flow present. Hence it is known that even at low air flow (0.150 sL min^{-1} for carbon dioxide and 0.400 sL min^{-1} for air) there is adequate lift of the growth medium to prevent back flow.

2.7 Photobioreactor preparation for use

The pH and temperature meter required calibration which was performed using *Reagecon buffers* at pH 4.00, 7.00 and 10.00 (± 0.01 at 25 °C). When the electrolyte solution required changing, the old solution was removed, replaced with fresh KCl 3 mol L^{-1} solution purchased from *Sartorius* and the pH meter recalibrated.

Sterilisation of growth medium (Bold's Basal medium) was achieved prior to addition to the photobioreactor by autoclaving in glass vessels sealed with aluminium foil; the algae inoculant was grown in medium which was also autoclaved. The photobioreactor was disinfected using 100 mL bleach, in sufficient water to fill the reactor completely, circulated for 20 minutes and then rinsed twice with deionised water for 20 minutes per cycle. Sterilisation was ceased to prevent contamination from the bleach affecting the growth of algae after a number of unsuccessful runs, however this was later determined not to be an issue and neither was non-sterilisation.

Table 2:2 – Photosynthetic photon flux in photobioreactor

Light type and colour	**Light pulsing regimen (light : dark)**	**Average photosynthetic photon flux / µmol photons m^{-2} s^{-1}**
White	Continuous	1.33
Red & blue	Pulsed (10 : 20 ms)	72.76
Red	Pulsed (10 : 20 ms)	44.84
Blue	Pulsed (10 : 20 ms)	37.13

The average photosynthetic photon flux was measured using a light meter (*LI-COR® 250A Light Meter*) using all of the appropriate LEDs or the two fluorescent lighting strips for white light the recorded light readings were acquired (Table 2:2).

2.8 Photobioreactor testing

2.8.1 Effect of flashing light on *Chlorella emersonii* growth

Under intensely bright light, it is reported that algae cells form smaller chlorophyll antenna and less chlorophyll a to protect them from photoinhibition; thus lowering photon absorption.[11] As introduced in section 1.5.13, 1.5.14 and 1.5.15 the use of pulsed light to combat oversaturation and photoinhibition has been postulated in literature.[1a] The pulse generators were set with a pulse width of 10 ms (100 Hz) and a period of 30 ms, thus giving an illuminated time of 10 ms and a dark time of 20 ms; as suggested by Gordon and Polle[1a] in the literature. This was set and measured using an digital storage oscilloscope, *Tektronix TDS2014B 100 MHz 4 channel*. The waveform and length was captured by the oscilloscope, then adjusted and tuned using the pulse generators until the desired pulse width and period was given. This was achieved for both the red and blue pulse generators.

2.8.2 Preliminary testing

For each growth test 9 L Bold's Basal solution was made up and autoclaved (see sections 4.9 and 4.11) 7 L for each run and the excess to maintain the 7 L level when run for multiple weeks. Algae for inoculation was grown as described in section 4.11 and used for inoculating the photobioreactor.

The photobioreactor was set up as described in section 2.7 – 'Photobioreactor preparation for use' with the carbon dioxide and air outputs checked and ensured to be consistent throughout each testing. The LED strips were checked strip by strip prior to the start of each testing to ensure they were illuminated as desired. The first few experimental runs were conducted with natural light due to the illuminated section being under construction, these appeared to allow slight growth of the algae, but true observation of change was difficult due to the small amount of algae yielded. These runs were useful to alter the photobioreactor mechanically to work optimally and without leaks. The initial 6 LED runs were carried out with full red and blue (2:1 ratio) LEDs pulsing at a pulse regimen of pulse width - 10 ms (100 Hz) and period - 30 ms (2.8.1 – 'Effect of flashing light on *Chlorella emersonii* growth'). Since none of the experiments yielded any algae growth the blue lights remained unlit for 4 follow up (red only - pulsed) experimental runs. There remained no quantifiable growth, thus the LED illuminating tower was removed and two white strip lights (obtained from University Central Stores) were added to the outer scaffold and the photobioreactor was modified to mimic successful vertical reactors in the Department of Biology, *University of Bath* growth rooms. The photobioreactor was filled with 3 L Bold's Basal growth medium to create a vertical airlift system.

The modification to white continuous light and to a vertical airlift reactor achieved slight visually observable growth at the top of the vertical section, mainly against the glass, some flocculation had occurred (repeated 4 times), though there was incorrect mixing to keep the algae flowing due to the change in configuration of the reactor. In this configuration the algae were concentrated at the top of the vertical column and once it agglomerated to saturation it dropped through the up flow of air in the algae collected at the bottom of the reactor where it was unaffected by the gas flow. This was successful for only 4 – 5 days however and adequate growth for cell counts or FAME profiling was not obtained even after 3 weeks.

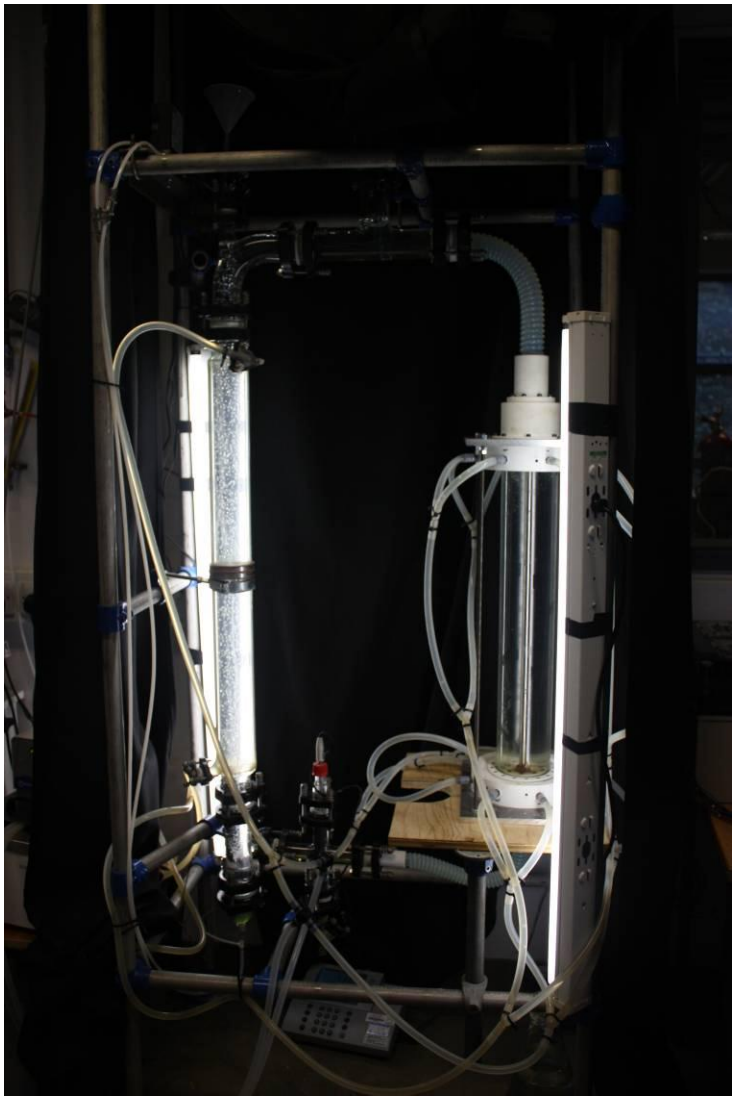


Figure 2:20 – Strip light photobioreactor illumination for white light experimentation: 2 white fluorescent tubes attached to the scaffold with the LEDs and support removed to allow light penetration to the algae biomass

The photobioreactor was then reassembled in its previous form of circular continuous airlift reactor to allow for further growth of the algae post 4 or 5 days and re-inoculated. In order to achieve this, the illuminating LED tower was temporarily removed to allow maximal white light saturation over maximum area of the photobioreactor (Figure 2:20). This was also run multiple times (6 times) at varying gas flow rates yet did not yield any quantifiable algal growth. Despite lowering of the flow rate of gas from over 2.5 sL min^{-1} to 0.5 sL min^{-1} there was no growth of algae within the system. Regardless of inoculation culture amount, concentration or maturity there was no observed growth throughout all experimentations. Each run was maintained for between 2 – 3 weeks to allow for acclimatisation of the algae in the photobioreactor.

2.8.3 Photobioreactor and algal growth issues

On-going throughout much of the experimentation phase, leaking was prevalent which led to the multiple repeat experiments carried out under each light regimen. These issues are explained in this section.

In phase one of the reactor design the bellows (Figure 2:21) presented a major leaking issue despite them providing the only flexibility within the airlift section of the reactor. The only way to completely seal this leak was to use silicone gel whilst still giving the movement required. Once the illuminated section was attached to the photobioreactor there were issues with leakage from the connecting sections of the airlift section of the reactor to the illuminated section due to rigidity within the design. The gap and alignment of the sections where the space for the illuminated section was to be placed was not as reported in *QVF* company literature and was distorted. This led to the 90 ^{o} bends being replaced in these sections by flexible tubing, which consequently required elimination of the bellows (Figure 2:21) and a switching of where the inlet valve was placed.

The replacement for the 90 ^{o} bends was required to be made from either glass or PTFE, since they would be in constant and prolonged contact with the algae biomass as well as to provide the flexibility to prevent further leaks. Glass was not an option due to its inflexibility and so PFTE flexible tubing was sought and found. Adjustment in all directions was required for complete sealing necessitating the use of flexible tubing to provide maximum options for repositioning. This was a relativity new product onto the open market and so there were no prior contacts, or known suppliers. After contacting numerous suppliers of tubing and consultation with the construction engineer of the illuminated section, it was decided that further end sections produced from glass filled PTFE to allow for joining of the flexible tubing to the glass airlift sections and illuminated section were required. Glass filled PTFE was also a relatively unknown material prior to the construction of this photobioreactor for the technicians involved. Once attached and the airlift reactor parts were moved to improved locations within the photobioreactor there were minimal issues with leaks. These leaks were due to gravity effects underneath the illuminated sections and thus aquarium-grade silicone sealant was employed to seal these leaks and to counteract the effects of gravity and biomass flow.

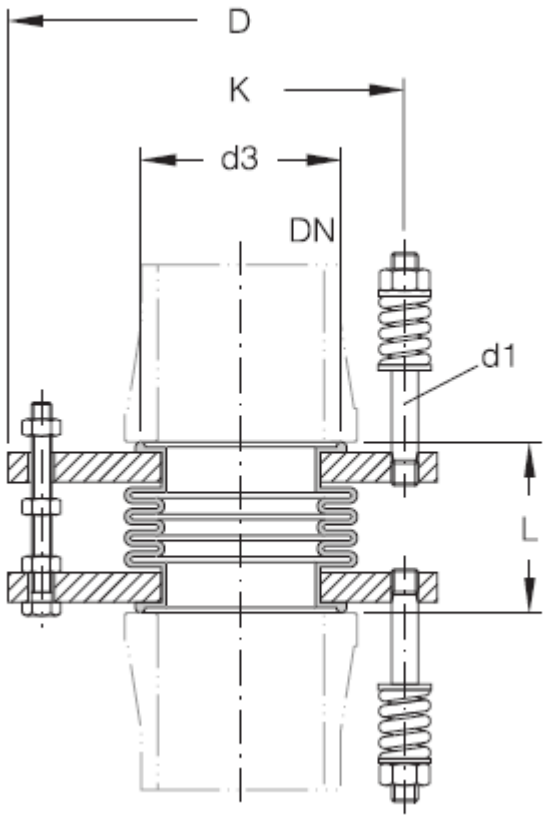


Figure 2:21 – Leaking occurred from the above bellows, which was the only point of flexibility within the system, they were sealed successfully using silicon rubber, however their removal upon the addition of the flexible PTFE tubing ensured that there was eradication of all leaks within the photobioreactor

Leaking occurred from the inner cylinder of cooling water to the algae passage via the top seal (see Figure 2:12 inner 80 mm glass cylinder). O rings had been designed and used for the seals within the end glass filled PTFE manifolds to ensure smooth contact between the glass cylinders and the manifolds. The end manifolds, due to their construction from glass filled PTFE, allowed for a little compression of the manifolds upon pressure. The PTFE seal being required to act to hold the glass cylinders in place was expected to act as a seal against water penetration too. The seal in this section was against the flow of water, thus allowing it to flow past at the pressure which the cooling water was pumped (due to small exit holes for the water causing the pressure build up). Since this seal was in contact with the algae biomass, whilst the contact is minimal, it was desired that there should be no compounds used for the sealing which would inhibit algae growth. The rubber seals used for the outer and inner glass tube were reported to inhibit algal growth, but were in contact with cooling water only. Holes to allow leaking water to escape were also drilled into the end manifolds to reduce the possibility of water leaking onto the electronic component of the illuminated section; these never became flooded during photobioreactor utilisation.

The use of two *Viton*® seals in replacement of the PTFE 'O' ring was tried to stop leaking between both outer and central cylinders and central and inner cylinders, whilst this slowed the leakage, it was not completely successful. Due to deformation the 'O' rings required replacement each time the illuminated section of the photobioreactor was dismantled for cleaning. In an attempt to combat this issue a different angle was then taken using restriction of cooling water flow.

If the flow from the cooling water bath was restricted (separately to the inner cylinder and to the outer cylinder) using 3-way taps/valves, the overflow flowed directly back to the water bath, this led to a lower pressure upon the seals. The valves required monitoring to maintain adequate pressure of the cooling water to the cylinders. Using water level monitors the 3-way tap/valve could be adjusted to the correct flow rate which maintained a level of water just below the seal, thus removing the seal from exposure to high pressure. This set-up was unsuccessful and was hence removed from the system.

The water was fed from the top to reduce pressure on the top seal, this initially led to leaks at the bottom seal, however with adequate control of water entering, and the reduction in pressure this led to, the seal at the bottom was expected to no longer leak due to being gravity sealed. This technique was unsuccessful after running cooling water for two hours.

Since none of the above methods were successful the use of PTFE foam to seal the troublesome area was trialled. This was cut into two lengths of 300 mm (diameter of section to be sealed) and each was cut to 4 mm width. This was partially successful to seal the middle glass tube for four preliminary tests. The PTFE foam required multiple applications and testing, however, and so was not a long term solution. It was therefore decided that a synthetic neoprene rubber would be trialled to permanently seal the leak regardless of its inhibitory effects on the algae growth, which are reported to diminish over time anyway (see Table 2:1).

Neoprene sponge (FOC) was the last material to be investigated for sealing the leaks between the outer cooling water and the algae biomass layer. Neoprene sponge was not initially used in this reactor design since it is a synthetic rubber and as such the effect of the subunits chloroprene are potentially very similar to rubber and isoprene which have

been shown to adversely affect algae growth. Due to continual leakage it was important that neoprene was not overlooked in the search for a sealant 'O' ring. The chemical inertness of neoprene gives it wide applications for gaskets, corrosion resistant coatings and hoses; it also resists burning better than exclusively hydrocarbon based rubbers. There was minimal contact of the algae species and the neoprene; however it has also been reported that over time the negative effects reduce. Neoprene sponge is resistant to air, ozone (which can be used to sterilise photobioreactors) and UV rays, it can also be safely used between -35 ^{o}C and 100 ^{o}C. The useful properties of neoprene sponge for this application were that it forms a waterproof seal whilst maintaining its flexibility as well as being fire resistant and thermally inert.

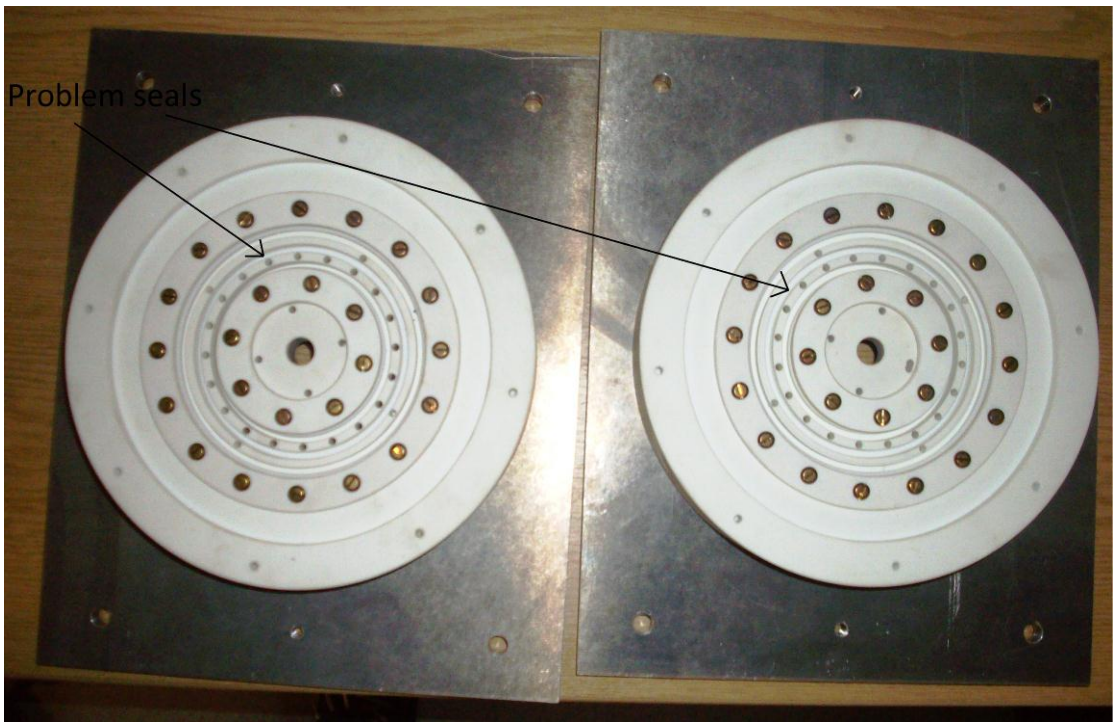


Figure 2:22 – Inlet and outlet end manifolds (as depicted in Figure 2:10) the glass filled PTFE manifolds were attached to steel plates for maximum stability: in the centre was the hole for the central steel support rod, and 4 steel support rods were attached for moving the illuminated section in the corners of the metal plates, the 20 holes for the algae to enter and exit the illuminated system can be observed, as can the attachment areas for the glass cylinders – the middle one which caused issues with leaking

Subsequent experiments proved that the neoprene seal was successful and its properties were of no issue to the algae, successful sealing of the middle glass tube was achieved (as highlighted in Figure 2:22). Growth of algae was unsuccessful with the photobioreactor even after the leaks from the seals were eliminated and some successful growth was obtained with the isolation of the airlift section from the illuminated section. This is suggestive of poisoning of the algae by one or more of the construction materials in this

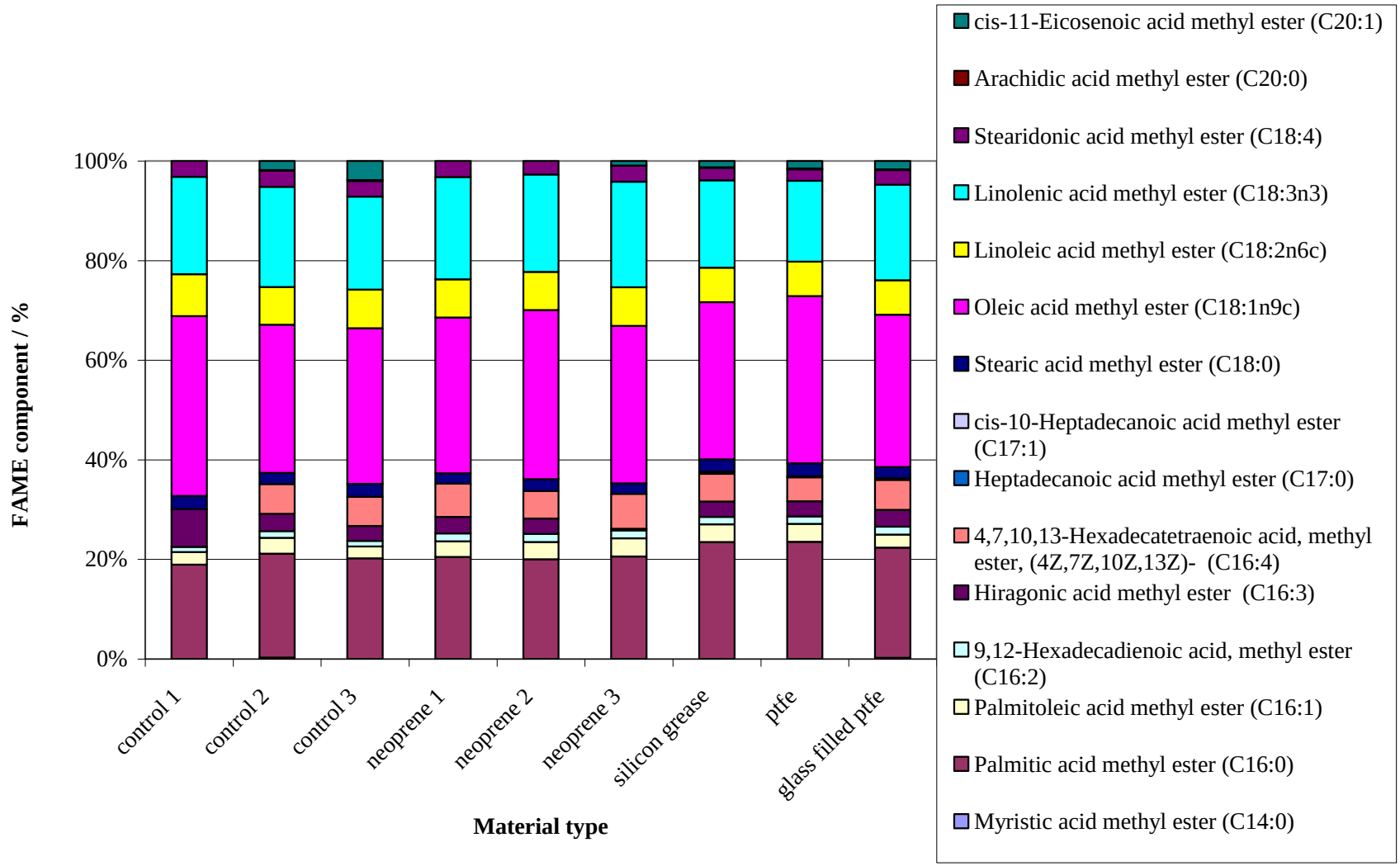


Figure 2:23 – Toxicity testing – FAME components from algae grown with materials from the photobioreactor (Table 2)

area or of toxicity by a sealing material. In order to discern which of the materials is affecting the growth so significantly; small scale algae toxicity tests were carried out in biology – Figure 2:23.

These toxicity tests showed no visual difference to growth with the presence or absence of varying amounts of neoprene, glass filled PTFE, PTFE and silicon grease. All of these construction materials permitted algae growth with no significant differences from the control cultures. Both overall growth and the FAME profiles are very similar for all scenarios tested.

Since there was no known cause of toxicity the photobioreactor was set up in full continuous flow mode and tested again at a much lower flow rate of 0.500 sL min^{-1}. Flow checks were carried out using air flow rates as low as 0.500 sL min^{-1} with resultant block flow, therefore this lower flow rate was used for experimentation. It was thought that there could have been potential shearing of the algae cells at the slightly higher flow rates or that the higher turbulence deters growth, hence reducing the flow rate.[9] Higher turbulence was observed within vertical reactors which does not inhibit growth, rather encouraging growth due to eddies being formed which mix the algae sufficiently and creates higher frequency light/dark cycles.[12] The benefits of turbulence are homogeneous mixing of nutrients and reduction of gaseous gradients, the prevention of sedimentation as well as the movement of algal cells through different light experiences.[13] To create more precise control over the input flow of the carbon dioxide and air, Swagelok taps were fitted upstream of the respective mass flow controllers; this allowed for adequate control and gas flow at the same rate as the successful vertical bubble columns used in the Department of Biology, *University of Bath* (section 2.6).

The electronic component of the photobioreactor design was produced in modular form to allow for replacement of LEDs and other maintenance issues to be achieved with ease. This was tested when the 5^{th} LED blue strip in the D section ceased to work. The strip was easily removed and replaced by the spare LED strip and a rogue washer was removed which had caused the blue LED strip to short. The LED strip was able to be tested and the LED which had blown was replaced. This proved that the modular design has been successful for the electronic section. It was also designed so that nothing in the control box would suffer damaged if there were issues with the LEDs or the strips, this would all

remain upstream of the control boxes, switching units and the power supply unit, again this was proved in practise when the strip shorted.

Testing for pathogens residual within the photobioreactor was then carried out. This involved taking Bold's Basal medium which had been resident in the photobioreactor for 1 month after inoculation with *Chlorella emersonii* which had been attempted unsuccessfully to be grown. This solution was taken to the Department of Biology, *University of Bath* growth room and two batches of 100 mL were placed in two 250 mL conical flasks, one of which was inoculated for a second time with 1 mL fresh algae. Both batches were placed in a growth room (at 25 ^{o}C with 300 μmoles s^{-1} light m^{-2} for 18 hours per day) for 7 days extra carbon dioxide was provided by sparging 3 % carbon dioxide in air through the samples. This proved successful for both batches of algae since both cultures grew; the solution re-inoculated with algae grew to 4.70 x 10^{6} cells mL^{-1} and the solution which had no new colonies of algae added grew to 4.00 x 10^{6} cells mL^{-1}. The cells in the re-inoculated batch were larger than those in the other sample; however both had recovered within 15 % of each other. This leads to the conclusion that there are no destructive compounds or pathogens present in the photobioreactor however there may be something in the gas supplies or in the atmosphere of the laboratory – despite having a closed system which uses bottled carbon dioxide and filtered air. The solution taken from the photobioreactor was also centrifuged and streaked onto LB-agar growth plates, with no antibacterial resistance. This allowed for the potential growth of fungi, bacteria or algae. There were bacterial and algae colonies present after 1 week of temperate (25 °C) growth; the algae could grow symbiotically with the bacteria present. This contaminant could be from the photobioreactor system, but is more likely to have come from the moving of the solution from the Department of Chemical Engineering to the Department of Biology, or from the centrifugation techniques employed or from the plating up despite the aseptic techniques employed. Though aseptic techniques and care was taken, this small study was to ascertain whether any bacterial, fungal or other algal colonies present were outcompeting or destroying *Chlorella emersonii* algal growth, hence no autoclaving was undertaken for this testing and the algal cells were able to proliferate satisfactorily. The algal cultures from the photobioreactor were still viable and able to proliferate once removed from the photobioreactor.

It was postulated that the non-growth of the algae may be due to chemical compounds in the air supply from other reactions and experiments occurring within the laboratory setting. This hypothesis was further tested by taking inoculated media which had been used in the photobioreactor and yielded no growth of algae, even after an acclimatisation period of 30 days. The inoculated media were removed from the photobioreactor and four batches of 250 mL were placed into four 500 mL conical flasks; two of these were inoculated further with *Chlorella emersonii*. All four batches grew algae in the biological growth rooms, with no supplementary gas added, and manual stirring daily over 10 days. Hence the conclusion of particulates or vapour presence in the air supply that was contaminating the photobioreactor. To combat the non-growth of algae, a gas inline filter was attached to clean the air and carbon dioxide entering the closed photobioreactor system. The filter acquired was a *vacu-guard^{TM} 150* which contains an activated carbon chemical trap and hydrophobic PTFE filter. The aim was to clean the compressed air coming from the laboratory system and to clean the bottled carbon dioxide.

Figure 2:24 – Sparger with air and carbon dioxide testing within the photobioreactor scaffold structure using the gas supply which was used for the photobioreactor

The addition of the inline gas filter only slightly encouraged algal growth with white continuous light. Since this small, non-quantifiable, improvement was observed it was decided that the sparger and gas supplies should be used to observe the growth of an algae

sample in the same position as the photobioreactor. The sample chosen had a significant and successful light history in the growth rooms. A 250 mL conical flask with 40 mL of Bold's Basal medium with a healthy *Chlorella emersonii* culture was taken; 60 mL of fresh media was added under sterile conditions. The culture was then moved to the Department of Chemical Engineering and the sparger used *in situ* to input the same internal air supply and carbon dioxide gas cylinder set up which is used in the photobioreactor (Figure 2:24). This lead to a healthy growing culture, thus the air and carbon dioxide set-ups were not thought to be causing the non-growth. (Initial culture cell count – 102 901 cells mL^{-1}; after 6 days growth – 378 543 cells mL^{-1} an increase in cell number by 3.7 times. A *Millipore Guava easycyte* flow cytometer was used for these cell counts).

Despite the gases being uncontaminated and the materials used for construction of the photobioreactor being non-toxic to *Chlorella emersonii* there remained non-growth during full system testing; independent of whether white light, red, blue or red: blue LEDs were utilised. The system without the specifically designed illuminated section was attempted to be used with the LED lights after some success with white light when the left hand side of the glass photobioreactor section, as detailed earlier in this section, though white light algal growth was not convincingly maintained. Fixing of the LEDs to the left hand side of the photobioreactor was unsuccessful due to the cooling water entry and exit points.

2.9 Alternative airlift vertical reactor for testing of LED illumination; Chlorella emersonii growth dependence on wavelength

Since the photobioreactor system was unable to grow quantifiable levels of algal biomass the system was disregarded and an "off the shelf" system was tested using the LED system. The "off the shelf" system had been developed and manufactured 3 years after the start of the design of the photobioreactor described in the above section. The testing of the LEDs was able to be carried out using an identical vertical reactor to those which have been proven to be successful for algae growth in the Department of Biology, *University of Bath*, with white continuous light in a set-up detailed in Figure 2:26. This ensured that the LED lights could be tested with no other light interference, as well as the air and carbon dioxide being the same. The temperature of the LEDs was not an issue, which was surprising since they release a lot of heat when all illuminated. The gap between the LED support and the

algae culture proved sufficient to allow for little heat energy transfer whilst being close enough for satisfactory light penetration. 7 L of growth medium was used per experiment. The successful growth after two weeks of culture of *Chlorella emersonii* in such a set-up with both red and blue light (2: 1 ratio) was comparable to that of the algae growth obtained in the white light of the growth rooms in the Department of Biology. This was corroborated by a second study using red and blue light in a ratio of 2:1 at a pulse width of 10 ms and a dark period of 20 ms. In conclusion, perhaps the entry and exit holes (20 x 3.5 mm) which the algae were forced through were too small thus causing cell shearing at the point of entry and exit to the illuminated section.[14] Steady laminar flow through channels can cause shear stress which stimulates cellular responses which can be unpredictable until studied for specific cells under each situation. Shear stress is caused by the frictional force of a fluid passing through a channel, the smaller the channel the higher the friction. Shear stress can stimulate the release of other substances by the cell which could be responsible for cell death due to changes in gene expression, cell morphology and metabolism.[15]

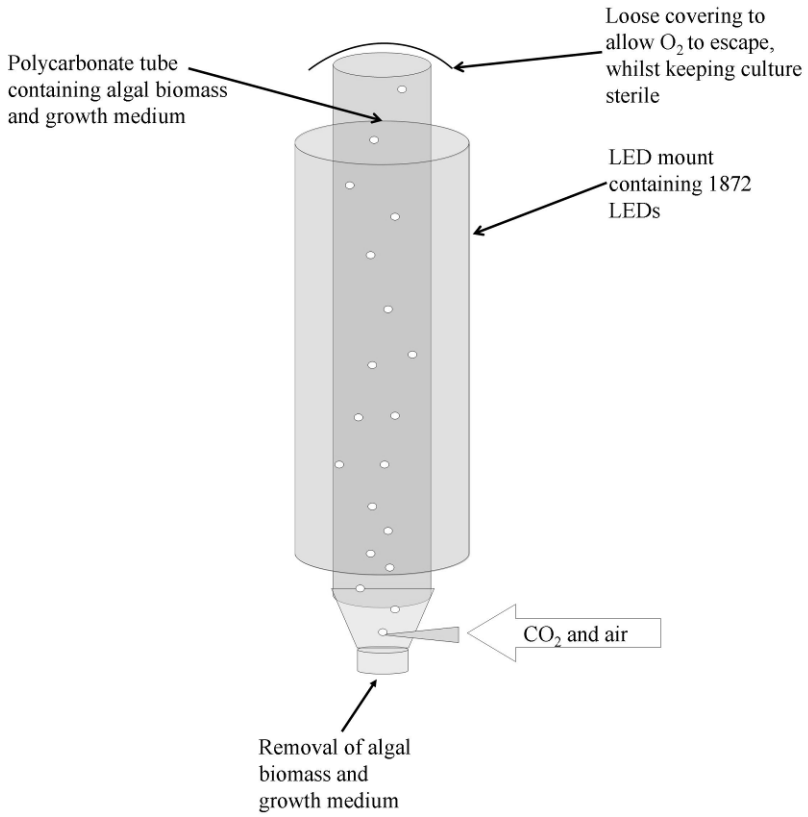
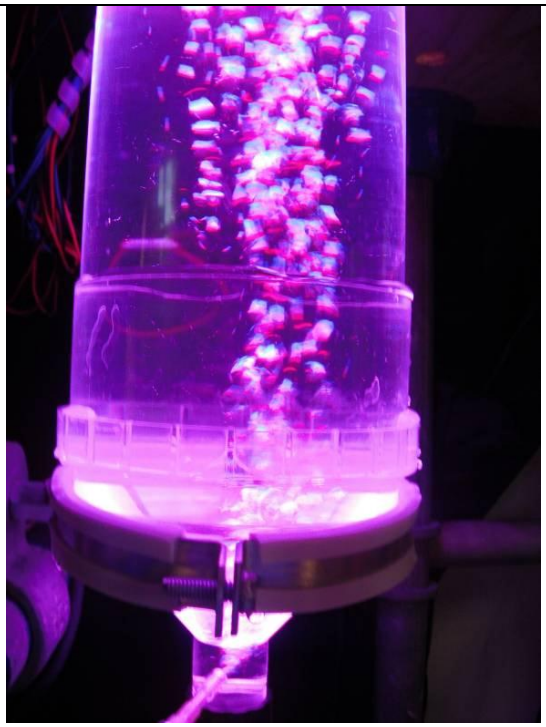


Figure 2:25 – Current set-up of pulsing LED photobioreactor: a vertical simplistic polycarbonate tube with an entry point for carbon dioxide and air (Figure 2:26c), a loose covering for the entry of growth media and algae (Figure 2:26b), the removal of algal biomass and growth media was via a sandwich bag clip at the bottom of the vertical column (Figure 2:26a); the LED section fitted around this design with no alteration required (Figure 2:26b&d)

A

b



C

d

Figure 2:26 – Final configuration of the photobioreactor: (a) gas entry point and tap off for algae culture; (b) illuminated LEDs with vertical column; (c) action of airlift with LEDs illuminated; (d) illuminated LEDs close up (both red and blue illuminated)

The sole use of red light did not allow for algal growth, it has been suggested in literature that red and far red light is required for photosystem 1 and red light photosystem 2 to progress[16] whilst supplementary blue light is reported to be essential for photosystem 2.[17] The photosystem 2 reactions allow cellular processes to occur such as function of enzymes and gene transcription regulation.[17-18] The energy derivation from exposure of algae to blue light is also critical to growth. It has been reported that the damage to cells by illuminating with solely red light is reversible by the addition of low levels of blue light for *Nannochloropsis* spp. and *Chlorella pyrenoidosa*.[17-18] This phenomenon of algal recovery was observed, after 2 weeks of pulsed red light growth was arrested. At this 2 week point, the blue pulsing LEDs were switched on at a 2: 1 ratio with the red pulsing LEDs. Growth was observed which was comparable with the growth of *Chlorella emersonii* observed under white continuous light in the Department of Biology, *University of Bath*. This was of particular interest since the algae had been revived from their dormant state merely by the addition of blue pulsed light. (Also notably, the additional carbon dioxide source was not available for the second part of this experiment (with the addition of blue light), thus there was potentially higher growth to be obtained, since conditions were not optimal). See Table 2:3 for an overview on *Chlorella emersonii* culture growth in the modified photobioreactor.

The cell count over time can be seen in Figure 2:27 for four of the different light conditions; red and blue pulsed together had the highest cell count after 14 days; continuous white light has a similar cell count to the pulsed red and blue light but appears to reach peak cell density at day 12. As can be observed in Figure 2:27, the use of blue light led to the lowest successful growth of algae, whilst adding in just $1/12^{th}$ of red light (i.e. a ratio of red: blue of 1: 12) led to an increase in cell count after 12 days. The use of solely red light was unsuccessful, as mentioned previously, for the growth of *Chlorella emersonii*. The increase in cell proliferation for the red and blue light (2: 1) pulsed at 10: 20 ms light: dark upon repetition showed how light history of the algae is important. The algae used in the second of these experiments had experienced a pulsed light history and therefore had acclimatised to the growth conditions already. Under growth conditions where the light history of the algae was different to that of the growth conditions being tested, lower specific growth rates were observed. See sections 1.5.11, 1.5.14 and 1.5.16 for more on light history.[19]

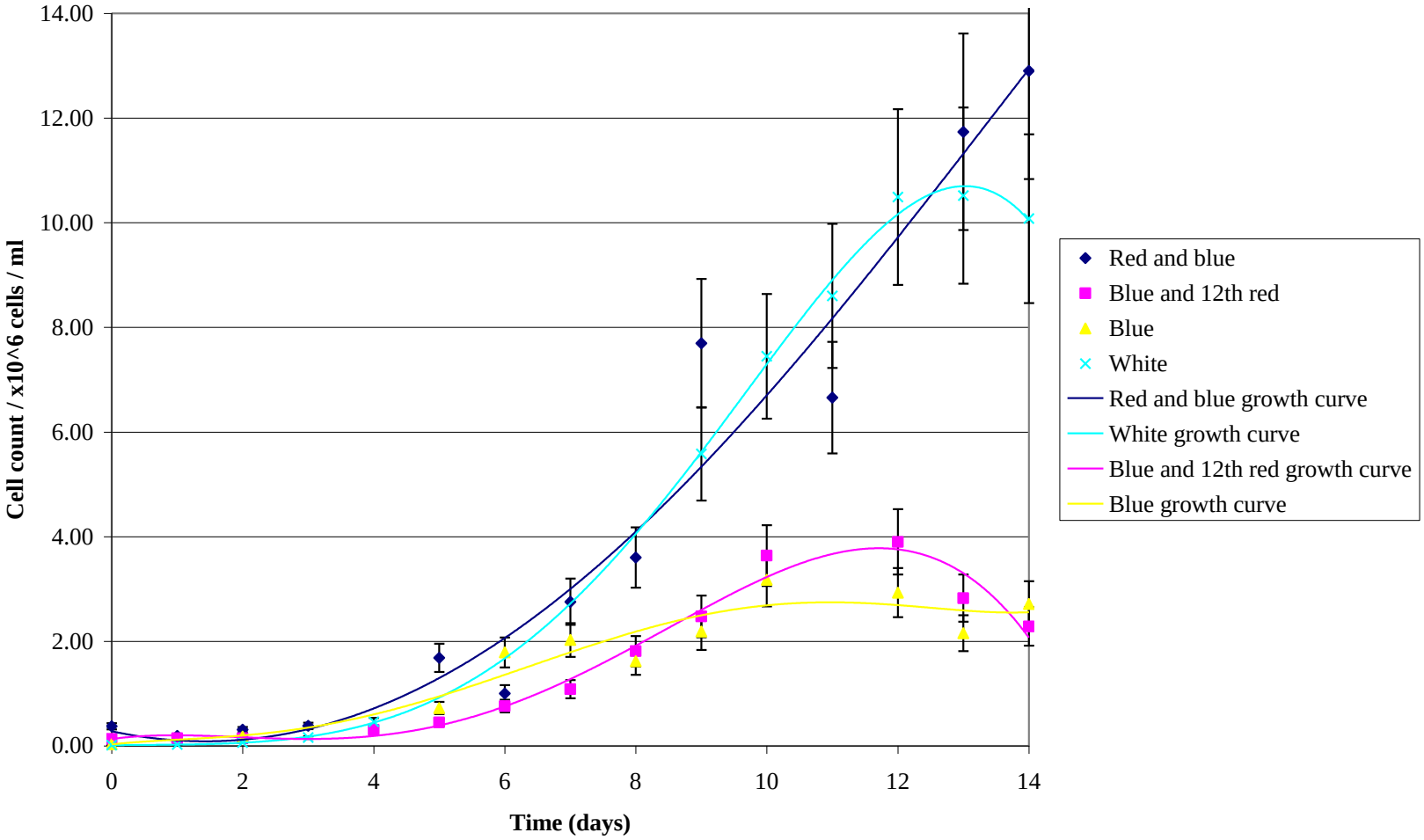


Figure 2:27 – Growth curves for *Chlorella emersonii* grown under varying light conditions in the vertical LED photobioreactor: dark blue – red and blue pulsed light; pink – blue and 1/12th red pulsed light; yellow – blue pulsed light; turquoise – white continuous light (Tables 3, 4, 5, 6)

Table 2:3 – Light impact on *Chlorella emersonii* growth

Growth light conditions (2 weeks)	**Cell count / cells mL^{-1}**
Continuous white light – white history	10 078 400
Red and blue light (2: 1) pulsed – white history	5 664 550
Red and blue light (2: 1) pulsed – pulsed history	12 900 000
Red pulsed light for 2 weeks, then red and blue light (2: 1) pulsed for 2 weeks – pulsed history	6 547 500
Blue pulsed light, with $1/12^{th}$ red pulsed light – pulsed history	2 282 500
Blue pulsed light – pulsed history	2 712 500
Red pulsed light (duplicated) – pulsed history	No growth

The growth of algae in the modified vertical photobioreactor with solely blue light was successful; although cell counts were lower for blue illuminated *Chlorella emersonii* the light intensity of the blue light was reduced (see Figure 2:28). If equal intensities of blue light and white light were used there could be some interesting cell counts observed. El Khachia *et al.*[20] observed that growth with modulated blue LEDs was possible for *Chlorella emersonii* and that it grew equally well in comparison to white light provided the intensity was high enough that all the photosystem reaction centres were excited.

The advantage of *Chlorella emersonii* being proven to be able to utilise pulsed light of specific wavelengths is the reduction in energy used to supply these specific requirements of the algae. Pulse widths used in the experiments resulted in one third of the energy requirements being used of continuous red and blue light. If the energy reduction of using just red and blue light instead of the complete spectrum is taken into consideration also, this energy saving would be even more significant for energy reduction. If incremental light intensity is also used, so that excess light is not outputted when the algal culture is more light penetrable, the energy required for algae growth could be further reduced. Das *et al.* observed that using incremental lighting reduced the energy input of lighting by up to 20 % compared to continuous illumination.[17]

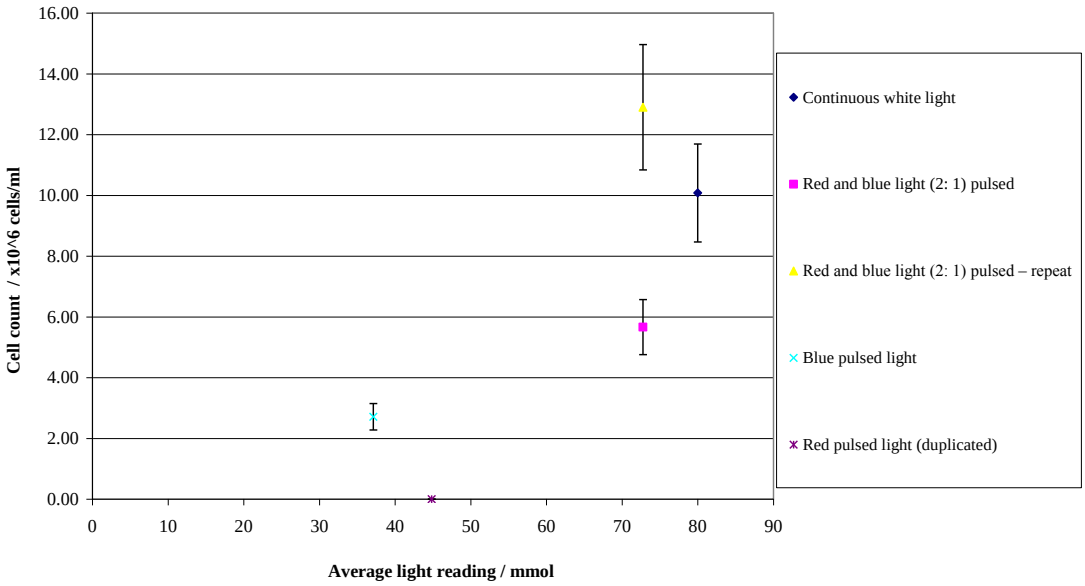


Figure 2:28 – Effect of light intensity and wavelength on algal growth (Table 7)

It has been observed elsewhere for the growth of *Chlorella pyrenoidosa* that monochromatic red light is able to support algal growth.[17, 21] It therefore appears that the algal cells sensitivity and requirement for specific wavelengths of light varies from species to species. In other literature, it has been observed that the lipid profile in *Chlorella* spp. varies with respect to light wavelength suggesting that lipid concentration and profile can be manipulated by the wavelength of irradiation.[22]

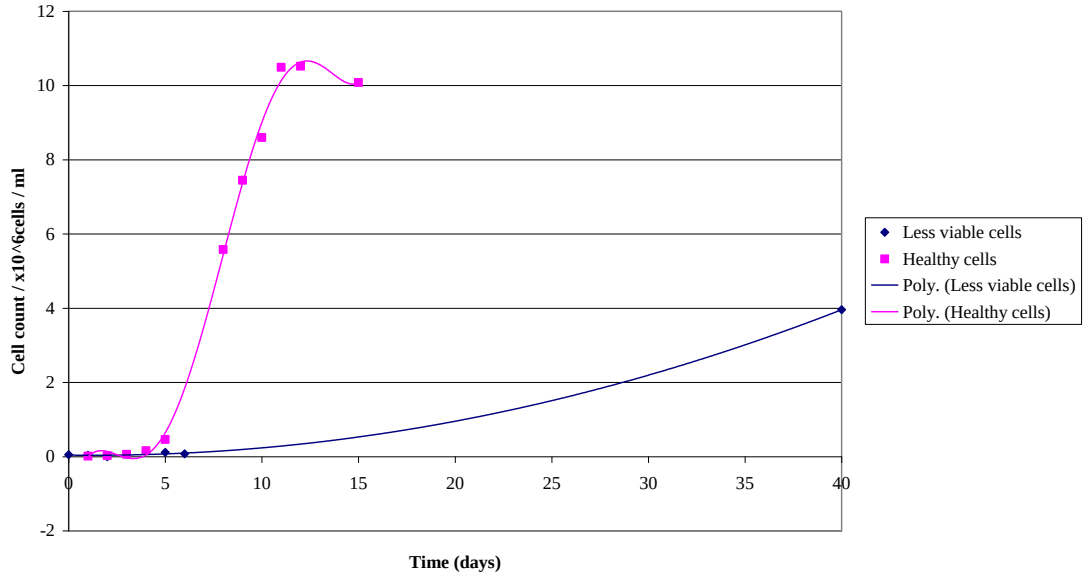


Figure 2:29 – Growth curve for viable and non-viable *Chlorella emersonii:* pink squares – viable cells and blue diamonds – non-viable cells (white light, 25 ^{o}C, Bold's Basal solution, 3 % carbon dioxide – Table 8)

The growth of cells which had already entered the decline phase of their life cycle can be used for inoculation, however as shown in Figure 2:29 (blue diamonds) the time required for recuperation and rejuvenation of cells is extended and higher growth rates are not observed (blue curve compared to 'normal' pink squares). It is therefore concluded that viable cells, in the exponential stage of their growth cycle should be used for inoculation.

2.9.1 Effect of light concentration on lipid profile and amount

The effect of light intensity and the wavelength used to cultivate the *Chlorella emersonii* algae can be observed in Figure 2:28. The use of continuous white light was taken as standard conditions; under these conditions there was a day: night cycle of 18: 6 hours. The continuous white light yielded around 10 million cells mL^{-1}. In the first study using red and blue pulsed light at a 2:1 ratio with a light:dark ratio of 10: 20 ms there was a reduction in algal growth to around 6 million cells mL^{-1}. There was, however, an increase upon a repeat of this experiment to around 13 million cells mL^{-1} which could be accounted for by the use of inoculant from a batch which had been subject to pulsed conditions previously. This highlights, as mentioned in section 2.9, that the recent light history of the inoculant algae may be responsible for the responses of the culture to different light conditions. Since the inoculum for the initial pulsed experiment had not been light acclimatised this is expected to account for the lower cell count observed. Once the inoculum had acclimatised the growth rate improved to around 13 million cells mL^{-1} growth medium and this, along with the decrease in light period and wavelengths used, highlighted the effectiveness of a pulsed light history on algal growth. As mentioned in the previous section 2.9, the LEDs were only lit for one third of the time so there was less energy being used within each 24 hour period (a total of 8 hours for pulsed LEDs and 16 hours for continuous white light). There was also a reduction in energy used due to the use of red and blue light, and not the fuller spectrum of white light, hence another potential energy saving.

The *Chlorella emersonii* FAME profile under different light conditions can be seen in Figure 2:30. These results were obtained by the harvesting of the total algal culture, centrifugation of the mixture and subsequent drying of the wet biomass. The lipids were extracted from the algal biomass and transesterified, then analysed using GCMS/FID. The predominant FAMEs produced under LED conditions were C16(0), C16(4), C18(1), C18(3) and C18(4); there was also C16(1) and C18(2) present in notable amounts. In other

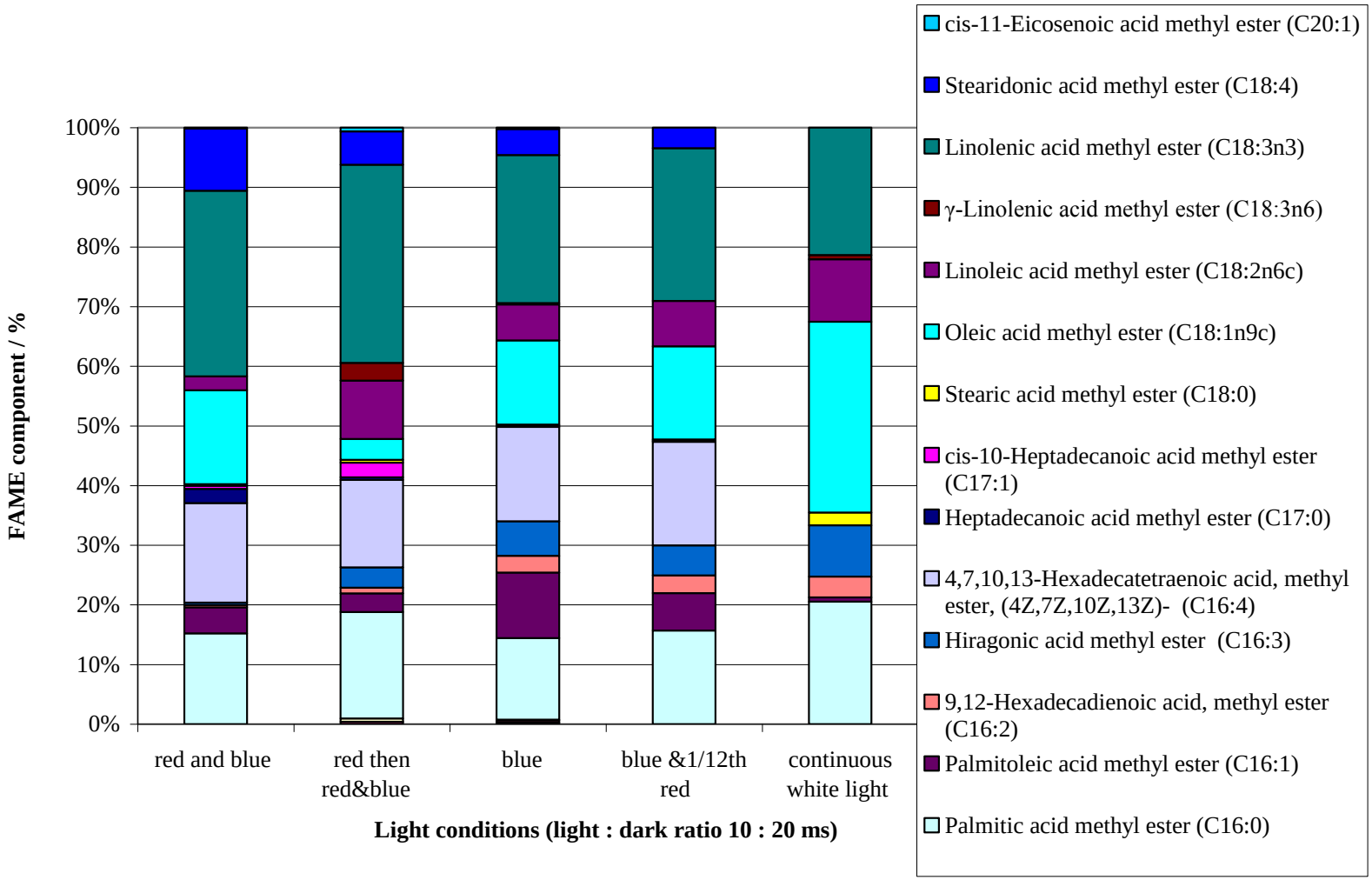


Figure 2:30 – Vertical LED photobioreactor effect on FAME composition: pulsed red and blue LEDs, 2 week growth; pulsed red LEDs for 2 weeks then pulsed red and blue LEDs, 2 weeks; pulsed blue LEDs, 2 weeks growth, pulsed blue and 1/12th red LEDs, 2 weeks growth,; continuous white light, 2 weeks growth (Table 9)

experiments using white light there was a dominance of C18(1) in samples from *Chlorella emersonii*; with C18(0) also being more abundant. Under blue light conditions, and predominantly blue light conditions there was an increase in C16(1) formation. It must be inferred that the light source and wavelengths impact the lipids and thus the FAMEs produced.

2.10 Summary

Life cycle analyses have indicated that photobioreactor technology has the potential to generate better positive energy returns compared to open ponds. Photobioreactors have advantages of process control and further improvement potential, however the energy balance between designs vary massively and little has been carried out on the optimisation of these designs. These provisos make life cycle analyses at this stage rather hypothetical and much needs to be done on improving process efficiencies in both open ponds and photobioreactors to realise the realistic viability of such technology.[23]

A unique photobioreactor was created which was unsuccessful in yielding algae. There was no growth under fluorescent continuous white light or under pulsed red and blue light. Initial testing required the eradication of leaks which was successfully obtained as described (sections 2.8.2 and 2.8.3). Systematic testing to determine the cause of non-growth of algae within the photobioreactor system followed: contamination from component parts of the photobioreactor was tested for and found not to be present; contamination from the air and carbon dioxide supply, as well as the possibility of other chemicals in the laboratory setting, were also tested – no issues with algal growth were found; finally to eliminate possible contamination by pathogens or other algal species, testing was carried out which excluded the presence of pathogens or competitive algal species within the system. The flow rate was also reduced from 2.5 sL min^{-1} to 0.550 sL min^{-1}, as described in sections 2.6 and 2.8.3, to prevent possible stress on the algal cells from fast flow and shearing this could have caused. In conclusion the non-proliferation of algal cells was likely to be caused by shear stress on the cells as they travelled through the 20 3.5 mm entry and 20 3.5 mm exit holes to the illuminated section (section 2.9).

Therefore an “off the shelf” tubular reactor was used to obtain results from the exclusive LED array; this new system incorporated a closed system with the unique light source

(pulsing LEDs), a carbon source (gaseous carbon dioxide), air, and to which nutrients could easily be added. The photobioreactor was designed and proved to be easy to clean so that the light path was not obstructed and included a removal tap for algal biomass and processed nutrients and waste growth media. The photobioreactor was designed to maximise algae growth, taking aspects from other reactors in the literature and had specific theoretical ideas incorporated within it. Typically incorporated into photobioreactor designs are the following features: light permeable illuminated area with short optical path from external illumination; natural, fluorescent, metal halide or LED lighting with photosynthetic range radiation (*i.e.* λ 400 – 700 nm); effective gas exchange (carbon dioxide and O_2); and easy to clean and harvest biomass.[24] Major limitations to algae growth in bioreactors and open ponds are suboptimal light supply, mixing and reduced biological efficiency, issues that this photobioreactor aimed to eliminate.

This photobioreactor incorporated aspects of LED technology which have been explored in literature by Gordon and Polle.[1a] The photobioreactor was designed to be flexible in its usage for adaptation for a variety of algal growth conditions. It was envisaged that the photobioreactor could be further developed for applications such as removal of carbon dioxide from flue gases and cleaning of municipal and commercial waste water. Solar cells and photovoltaics could be used to provide 24 hour illumination to the algae, or energy efficient batteries could be used to store energy to power the LEDs.

Since this photobioreactor was designed to investigate the effect of light on algal growth and the experimental set-up has not yet been optimised fully for *Chlorella emersonii* growth and adjustments were made to obtain measurements; manufacturing economics would not make commercialisation a possibility at the current stage. An up-scaled version of this photobioreactor designed specifically for *Chlorella emersonii* alongside particular light outputs would be required to compare this system economically with commercially available conventional systems; as such these comparisons are not justified. The resultant FAME profiles of the algal lipid yielded from experiments were able to be analysed for the specific wavelengths and pulse lengths tested.

The most rigorously tested reactors in literature particularly those with quantitative data are of 500 mL and below capacity; most testing has been carried out on cultures of around 50 mL; therefore the size of the photobioreactor tested in this thesis (7 L) is of a significantly

larger scale which can act as a useful interim stage for data collection and analysis before advancing to commercial scale photobioreactors.[17, 22, 25]

The use of bi-chromatic (660 nm and 470 nm) pulsed light at 10 ms pulse length and 30 ms pulse width was shown to be at least equal to continuous white light under the photobioreactor growth conditions. In comparison to the results, experiments by Vejrazka[26] detailed that a light: dark ratio of 0.1 using pulsed white light between 1 – 100 Hz (i.e. pulse length of 1 – 100 s) on an unnamed microalgal species led to a marked decrease in the specific growth rate and photosynthetic efficiency of the algae, compared to continuous white light. The use of 10 ms as pulse length and 30 ms as pulse width was chosen as hypothesised by Gordon and Polle[1a], the testing carried out within sections 2.9 and 2.9.1 proved the hypothesis that this would be comparable, and potentially superior to continuous white light. Studies in literature reported that white pulsing light in the order of tens of microseconds yielding higher bioproductivity of the algae[27]; similar studies for mono-chromatic or bi-chromatic light have not been carried out in literature. Most noticeably Phillips and Myers[28] in 1954 studied the effect of chopped white beam light at 1 ms (with non-defined dark stages) and recorded that the resultant growth of algae was the same as with continuous white light; later, in 2011, Xue[19b] reported the use of high frequency pulsed white LEDs (0.01 – 20 Hz) which gave variable changes in specific growth rate (variance was attributed to turbulence within the algal system).

The use of continuous blue light has been compared with continuous white light by Das *et al.*[17] and Perez-Pazos *et al.*[22] where it was found that blue light led to slightly increased biomass productivity of an unnamed *Chlorella* sp. especially when supplemented with $CaCO_3$. Other species of algae have been shown to increase in proliferation rates when solely red light has been used.[25d, e]

The use of pulsed red and blue light has not been studied in literature before; neither has direct comparison of continuous white light and pulsed mono-chromatic or bi-chromatic light from LEDs. For the experiments carried out in this thesis after 2 weeks of growth the continuous white light culture had entered into the stationary growth phase and cell death (Figure 1:7), whilst the pulsed bi-chromatic culture was continuing in the exponential growth phase; therefore the pulsed bi-chromatic light enabled higher cell counts to be reached. The FAME profile of the transesterified lipids in each culture were largely similar,

however there were higher degrees of unsaturation in the pulsed bi-chromatic culture compared with a higher percentage of saturated FAMEs and C18(1) produced under continuous white light. Literature records that there at higher light intensities there is generally an increase in overall lipid accumulation, especially of saturated triglycerides.[29] Tang *et al.*[30] have reported that there are no significant differences in FAME profile or percentage of FAME yielded from *Chlorella minutissima* regardless of white light: dark ratios on the hour timescale compared to continuous light; therefore it would appear that the timescale of the pulsing may not have an effect on the resultant FAME profile of the algal lipid, but the wavelength.

The growth rate of the algae under continuous white light and under the pulsed bi-chromatic light were similar (Figure 2:27); the pulsed bi-chromatic light used the light more economically than the continuous white light. This improvement in light economy was due to the pulsing of the light, which reduces the energy used by half (24 hour 2: 1 dark: light ratio *cf.* 18 hours continuous white light) and a further reduction by using discrete wavelengths and not the full spectrum (Figure 2:28). The comparable growth rate could also be attributed to the loss of biomass observed under prolonged dark periods, 6 hours, which is eliminated by having 24 hour illumination.[12a]

The use of solely pulsed red light for growth of *Chlorella emersonii* was found to be unsuccessful due to the requirements of photosystem II for blue light, and that subsequent addition of pulsed blue light revived the algae from their dormant state.[31] The lower energy requiring pulsed blue light enabled growth of *Chlorella emersonii* with minimal changes in FAME profile and lipid percentage, whilst supplementation of blue light with even $1/12^{th}$ of the amount of red LEDs as blue LEDs increased algal cell growth by around a third (Figure 2:27).

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3 Effect of environmental factors on fatty acid methyl esters composition in *Chlorella* spp.

Chapter three focuses on the environmental factors that affect *Chlorella emersonii* growth, particularly for maximisation of lipid yield and output of predictable, useful and reliable fatty acid methyl ester (FAME) profile. Lipid extraction via Soxhlet and microwave extraction as well as transesterification techniques were explored for this species of algae. FAME profiling was developed and determined using GCMS. The effects of nitrate, phosphates and iron concentration and culture time upon FAME profile and lipid yield were explored using a response surface methodology technique which allowed for the effects of two variables to be analysed for different outputs. The use of mixotrophic growth and the effect of different carbon sources on FAME profile were investigated too.

3.1 Lipid extraction

D'Oca *et al.*[1] reported that chloroform: methanol in a 2:1 ratio extracted the most lipid from *Chlorella* species. The use of chloroform: methanol in this ratio was also found to be successful for a number of species of algae by different research groups.[2] Cescut *et al.*[3] reported that using Soxhlet extraction equipment with 2:1 chloroform: methanol the composition of lipids extraction was 60 % polar and 40 % non-polar. Initial screening to extract maximal total lipid content from the algae using Soxhlet extraction techniques was carried out using a variety of solvents on *Chlorella vulgaris* algae samples from *University of West England* (UWE). A ratio of 2: 1 chloroform: methanol was found to extract lipids most successfully. Dichloromethane and diethyl ether extracted low amounts of lipid, 5.8 % and 4.5 % respectively. These reactions were ceased after 14 hours since there was no further visible extraction of the green pigment chlorophyll which is lipid soluble. For the tetrahydrofuran experiment there continued to be extraction of further chlorophyll until 62 hours suggesting that lipid was being continually extracted. Tetrahydrofuran (THF) required more time (4.4 times increase) for extraction but did extract an insignificantly higher percentage of lipid at 16.9 % compared to the chloroform: methanol mixture (13.0 ±1.98 %), however the 2: 1 mixture was chosen to be used in further experiments due to its ease of extraction, relative cost and time considerations. The use of methanol in the extraction phase simplified the process of biodiesel production as methanol was used in the transesterification step. This reduced the number of solvents and compounds used enabling

a more cohesive and economical production cycle. Higher lipid extractions were found for algae grown under low nitrogen conditions (52 % for *Chlorella emersonii* when extracted using chloroform: methanol), however the growth rate of algae was extremely slow under these stressed conditions. Table 3:1 shows the small (around 0.1 g where possible) total dry weight of sample used for initial extractions. The total lipid weight percentage value was approximate as a larger sample would be required for more accurate analysis to be carried out by mass. The percentage of total lipid compared favourably with reported values for lipid content in *Chlorella vulgaris* of 18 - 40 % (protein reduced from 29 % to 7 %) and for *Chlorella emersonii* of 29 – 63 % lipid content reported in literature.[4]

Table 3:1 – Initial total lipid extraction solvent tests

	Total weight (dry) / g	**Total lipid / weight %**	**Extraction time / hours**
THF	0.1391	16.9	62
Ether	0.1298	4.5	14
DCM	0.0908	5.8	14
$CHCl_3$/MeOH (average)	0.1154 ± 0.0430	13.0 ±1.98	14

The different solvents extracted varied amounts of lipid from the *Chlorella vulgaris* samples which were all taken from the same batch of algae. The remaining lipid must have been residual in the algal samples due to the solubility of the lipid in the different solvents as well as the differences in rates of solvent extraction. The lipid amount and the biodiesel profile of the algae is of utmost importance dictating the future application of the biofuel. The resulting biodiesel must have good oxidative stability, have identifiable cold flow properties and be of high ignition quality.[5]

3.2 NMR spectroscopy initial screening

Initial screening of different *Chlorella* spp. cultured at the *University of Bath* from cultures obtained from the *University of West England*. Different *Chlorella* spp. were looked at due to the fluctuation in lipid percentage, it has been reported in literature that the *Chlorella* genus generally produce higher amounts of lipid and are easier to cultivate than other genera. [6] The different *Chlorella* spp. produce varying amounts of lipid which depend also on growth conditions.[1] D'Oca *et al.* reported that *Chlorella vulgaris* produces 14 – 22 % lipid, whilst *Chlorella ellipsoida* gave 4.49 % and *Chlorella pyrenoidosa* 2 – 11.9 %[1]

therefore extraction was carried out with different solvents (Section 3.1) and species to determine which method (Table 3:1) and species (Table 3:2) to continue work with.

After initial lipid extractions using chloroform: methanol (2:1) Soxhlet extraction, the lipids were transesterified to produce biodiesel (sections 4.2 and 4.3). Acid catalysed transesterification reaction was chosen due to saponification with the alkali catalysed reaction and the cost, time and repeatability implications of heterogeneous catalysts on a small scale. Alkali catalysed reactions yielded water, which led to soap formation; thus sulfuric acid was used stoichiometrically to the extracted lipids. Acid catalysts are particularly useful when free fatty acids are present as they produce purer products. Miao and Wu[7] found the optimum stoichiometry for sulfuric acid conversion with *Chlorella protothecoides* was 100 mol%, hence this value was used for reactions.[8] Due to cost of alcohols, methanol is used; it was either used at a ratio of 6: 1 (to triglyceride) or in excess when the triglyceride could not be quantified adequately (20 mL was used as a standard amount to allow for adequate mixing for lipid samples which could not be quantified by weighing).

The extractions and subsequent transesterifications yielded the following FAME percentages and FAME profiles, as observed in preliminary tests by mass spectrometry (Table 3:2). The rather high standard deviations (Table 3:2) in the percentage of biodiesel produced are due to the small amounts of dried algae being used for comparison (< 0.1 g).

Table 3:2 – Biodiesel produced from preliminary tested algae from *UWE* – fatty acid methyl ester detected by mass spectrometry (pH 6.5 signifies the lowered pH due to a lowered nitrogen level)

Algae species	Biodiesel produced (%)	Fatty acid methyl esters present
Chlorella vulgaris	13.0 ± 1.98	C16(0), C18(1), C18(2), C18(3)
Chlorella emersonii	16.2 ± 4.5	C16(0), C16(1), C16(3), C16(4), C18(1), C18(3), C18(4), C20(0)
Chlorella emersonii (pH 6.5)	51.6 ± 12.0	C16(0), C16(1), C16(3), C16(4), C18(1), C18(3), C18(4), C20(0)

The percentages of biodiesel produced were determined by ^{1}H NMR spectroscopy (section 4.4 – method 2), using an internal standard (**Error! Reference source not found.**). The relative integrals of the benzaldehyde carbonyl peak (1H) at 10 ppm and the terminal methyl ester peak around 3.6 ppm (9H).

Fatty acid methyl esters obtained from the acid transesterification of the *Chlorella emersonii* baseline cultures grown at the *University of Bath* were C16(0), C16(1), C16(2), C16(3), C18(0), C18(1), C18(2), C18(3) and C22(5); whilst only C16(0), C18(1), C18(2) and C18(3) were detected for *Chlorella vulgaris*. High ratios of unsaturated fatty acids were present in the *Chlorella emersonii* samples taken; therefore this would produce a fuel with a lower cetane number which means it would potentially burn less completely and be susceptible to degradation. *Chlorella emersonii* had a fatty acid composition most similar to petroleum derived diesel and was thus most desirable. There was a higher percentage of lipid in the *Chlorella emersonii* samples and therefore biodiesel produced, whether under standard conditions or limited conditions. The combustion quality of this algal biodiesel was expected to be lower than for biodiesel with a higher proportion of saturated fatty acids, *e.g.* rapeseed however this would have required engine testing.

3.3 Fatty acid methyl ester profile determination using GCMS

Gas chromatography mass spectrometry (GCMS) was critical in obtaining quantitative FAME constituents, such as the one detailed below for standard *Chlorella emersonii* FAME profile (Figure 3:2), even for the small quantities obtained from <100 ml culture growth. The method developed is described in Section 4.6. The FAME composition was determined for biodiesel from various sources and algal species. As shown in Figure 3:1 there was a wide range of FAME compositions for biodiesel from different sources, coconut and palm oil derived biodiesel contained a higher percentage of shorter more saturated FAMEs.

For terrestrially sourced bi odiesel (i.e. all those apart from algal biodiesel) C16(4) and C18(4) were not present. However for algal biodiesel, *Chlorella emersonii*, there were fatty acid methyl esters derived with four degrees of unsaturation in the hydrocarbon backbone in varying amounts. Under standard growth conditions, as shown in Figure 3:2 and Figure 3:1, they are not present in values above 1 %, however they were detected (Table 3:3). This was corroborated by literature for algae that higher degrees of saturation were often seen, particularly C16(4) and C18(4) in green algae; interestingly different percentages of C16(4) and C18(4) were observed under various conditions.[1]

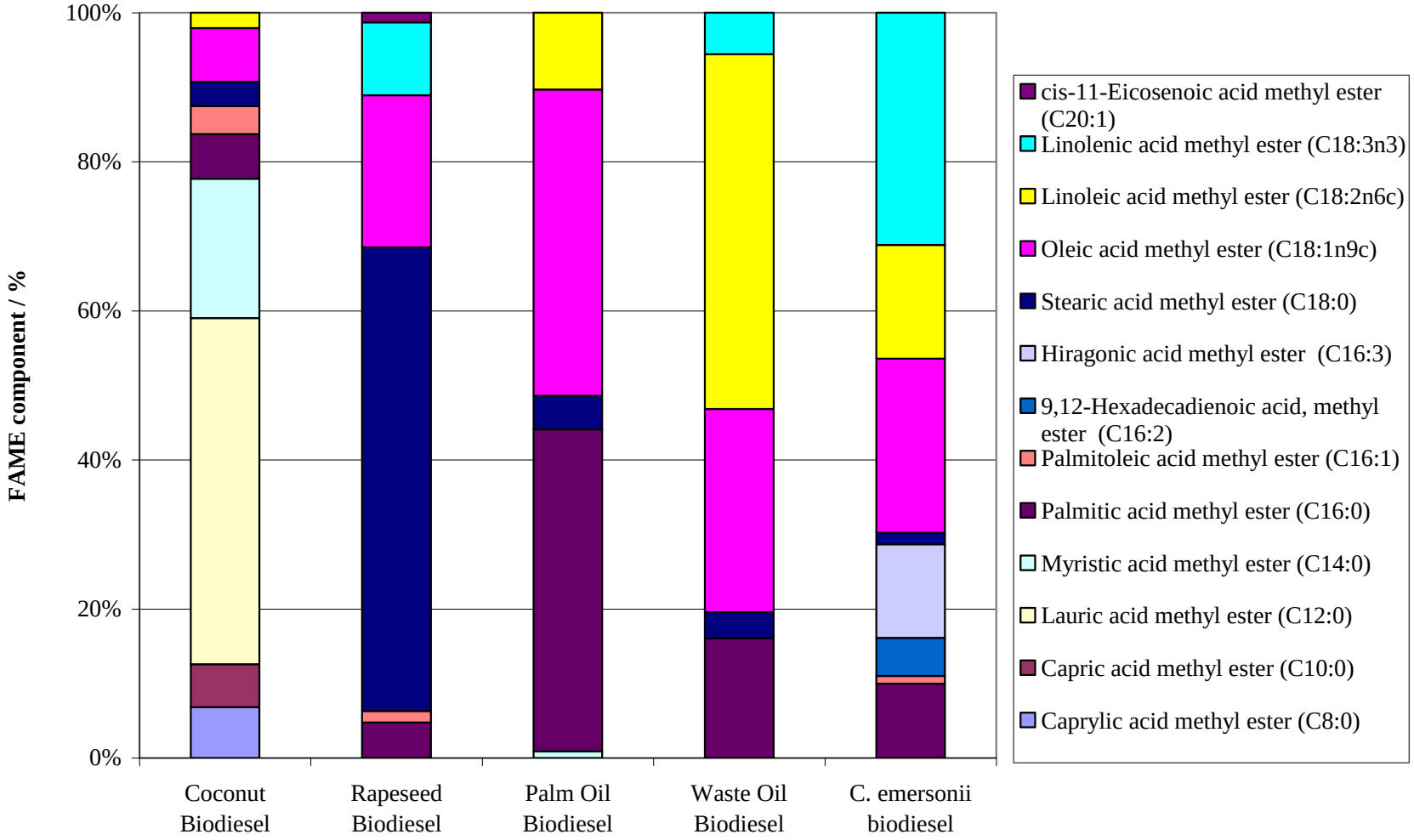

Figure 3:1 – Biodiesel composition from different sources: terrestrial and algae (Table 11)

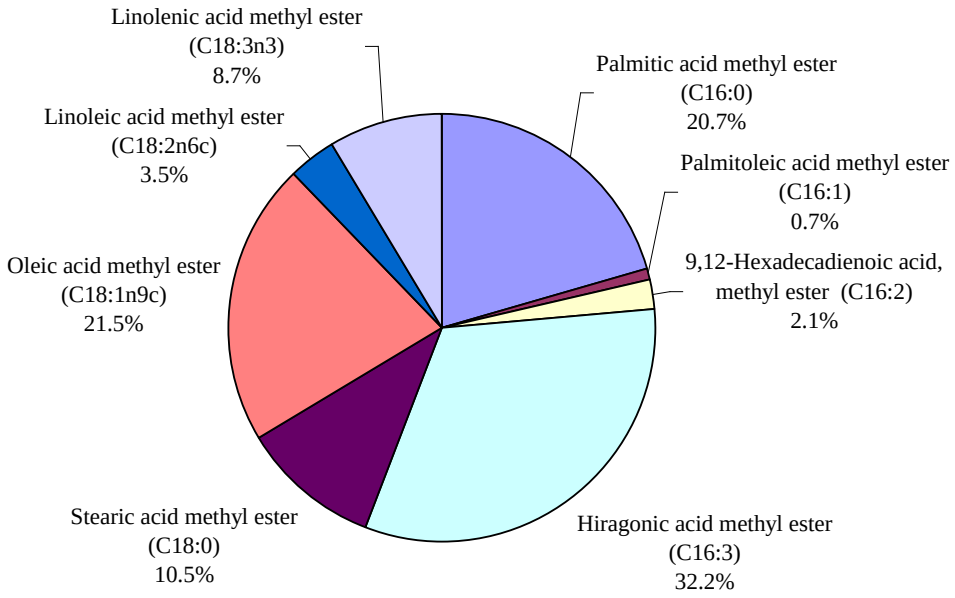

Figure 3:2 – Standard *Chlorella emersonii* constituents obtained from GC-MS results (see Section 1.2.2 for nomenclature explanation) (Table 10)

3.4 Effect of carbon dioxide concentration on algal growth

Optimisation of growth conditions to increase cell proliferation and lipid production was necessary for the scale-up of algae biofuel use. Carbon is required for photosynthetic algae growth in the form of carbon dioxide. It is reported in the literature that increasing the carbon dioxide concentration above atmospheric levels leads to accelerated growth of most algal species, including *Chlorella* spp., however too much carbon dioxide can have negative effects on cell growth.[9] For example, Fulke *et al.*[10] reached maximal *Chlorella* spp. growth at 3 % carbon dioxide whilst increasing levels to 10 % reduced algal growth to below that which was observed with 0.03 % carbon dioxide present.

Due to initial poor growth preliminary tests, in growth room experiments and in the airlift reactor, it was proved that when carbon dioxide enriched air was bubbled at any concentration through the growth medium a dramatic increase of growth was observed. This has been corroborated in literature, for example, Amaro *et al.*[11] who observed that *Nannochloropsis oculata, Scenedesmus obliquus* and *Chlorella kessleri* all exhibited a notable increase in biomass and lipid content with addition of carbon dioxide in air. It has also been reported that the residence time of the carbon dioxide needs to be sufficient to allow adequate time for mass transfer to occur. Due to these results and literature, as mentioned above and in sections 1.3.6 and 1.3.7, the increased growth rates observed led to

consequential studies using carbon dioxide enriched compressed air which was flowed into the algae growth vessels at 3 %. (For photobioreactor experiments 5 % was used due to the set-up and the flow rate required).

Table 3:3 – Minor fatty acid methyl esters present in some samples of *Chlorella emersonii* biodiesel

Caprylic acid methyl ester (C8:0)	Heneicosanoic acid methyl ester (C21:0)
Tridecanoic acid methyl ester (C13:0)	cis-8,11,14-Eicosatrienoic acid methyl ester (C20:3n6)
Myristic acid methyl ester (C14:0)	Arachidonic acid methyl ester (C20:4n6)
Myristoleic acid methyl ester (C14:1)	cis-11,14,17-Eicosatrienoic acid methyl ester (C20:3n3)
Pentadecanoic acid methyl ester (C15:0)	Behenic acid methyl ester (C22:0)
cis-10-Pentadecenoic acid methyl ester (C15:1)	cis-5,8,11,14,17-Eicosapentaenoic acid methyl ester (C20:5n3)
Heptadecanoic acid methyl ester (C17:0)	Erucic acid methyl ester (C22:1n9)
cis-10-Heptadecanoic acid methyl ester (C17:1)	cis-13,16-Docosadienoic acid methyl ester (C22:2)
C17:3	Tricosanoic acid methyl ester (C23:0)
C17:4	Lignoceric acid methyl ester (C24:0)
Arachidic acid methyl ester (C20:0)	Nervonic acid methyl ester (C24:1)
cis-11-Eicosenoic acid methyl ester (C20:1)	cis-4,7,10,13,16,19-Docosahexaenoic acid methyl ester (C22:6n3)
cis-11,14-Eicosenoic acid methyl ester (C20:2)	

3.5 Design of experiments to study the effects of nitrate, phosphates and iron levels and time on lipid profile and algal growth

The effect of key nutrients of algal growth and viability is important and early on in the project there were improved lipid percentages obtained from algae grown under low nitrogen conditions from algal samples supplied by *UWE* (Table 3:2). The low nitrogen conditions lowered the growth rate of the algae but led to an increase in lipid production due to the stress conditions formed by having limited nutrient supply. To investigate the effects of stress conditions on algal growth, lipid production and subsequent FAME profile a set of experiments (Box-Behnken methodology) was designed to follow FAME profile with respect to time. Box-Behnken methodology allows the synergistic effects of two parameters to be studied. The effects of nitrate, phosphates and iron limitation were

assessed over time, as the nutrients become limited and eventually unavailable to the algal cells and also where they were in excess and how this affected growth and subsequent FAME profile.

Nitrate and phosphates are considered in literature to be required for healthy algae growth in significant quantities, therefore alongside the preliminary data that nitrate levels affected lipid percentage these appeared to be two key nutrients to look into further.[4, 12] Nitrate is essential for nucleic acid and protein development, whilst phosphate is essential for DNA.[13] Iron levels have been highlighted a little in literature as being absolutely essential for algal growth and seemed to be a good candidate for observing the effects of macronutrient limitation. Increased iron levels are also reported to give rise to increased lipid levels in some *Chlorella* spp..[11] See sections 1.3.5 and 1.3.10 for further information on nutrient effect on algal growth.

A response surface methodology, such as Box-Behnken experimental design[14], was required to observe changes in nutrients and time of growth and the effect on lipid output and consequent FAME composition.[14-15] The ability to analyse more than one output by using this method of design of experiments was desirable for effective and efficient screening of factors. The following multiple outputs were analysed: overall lipid production (measured as subsequent FAME produced), C18(1), C16(4) as well as saturates, monounsaturates and polyunsaturated FAMEs. The Box-Behnken methodology[14] was chosen due to its ability to allow the systematic study of multiple factors and their interactions; it has been used for growth media optimisation for other microbial applications in literature which is indicative of its suitability for such a study.[15] The response of algae to changing nutrient levels and other factors was known; however there were also interrelated synergetic factors which can affect algae growth and lipid accumulation.[4, 16] This was more relevant to real-life situations such as algae growth in wastewater where increased nitrates and phosphates are present.

For each variable, three equally spaced values were used and the effect of changing two variables (to observe any possible synergetic effects on the fatty acid chains of the triglycerides synthesised by the algae) at the same time were able to be monitored and therefore predicted, if everything else remains equal.[14] The use of computer statistical programming, *MATLAB*® model-based calibration design of experiments software,

allowed for the prediction of certain outcomes for FAME profile under specified conditions, along with predicted error.[14] The model for the resultant graphs depicting predicted C18(1), C16(4), monounsaturates, polyunsaturates, saturates and total subsequent FAME was generated within the *MATLAB*® environment and fitted using a radial basis function network (see the appendix).

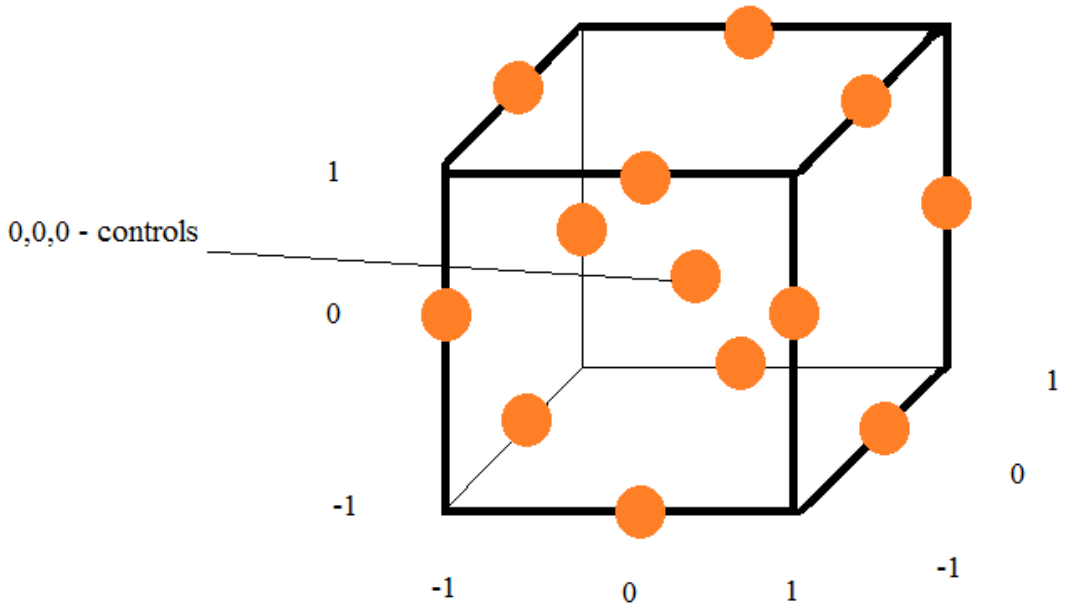

Figure 3:3 – Box-Behnken design (shown for three factors/variables); orange points are measured variables

The use of Box-Behnken methodology allowed for predictive models to be built by analysing the trends of the outputted data in response to the controlled input conditions. Other design of experiments were possible to allow analysis of such inputs, however they required more tests to be carried out and were more useful if there had been unknown/random expected outcomes. A total test maximum number of 27 was possible due to the equipment, space available and processing time required, since live samples were being manipulated and analysed. Box-Behnken design of experiments emphasised the ideal levels for each of the variables and was particularly useful when the optimum levels of the variables were expected in the centre of the experimental space, i.e. Bold's Basal medium. At the centre of each graph the prediction capabilities of Box-Behnken were strongest, with weaker predictions possible at the corners of the space. This was due to the use of just three levels for each variable or factor being used (see example Figure 3:3 for three variables – for simplicity); this allowed for fewer tests and was chosen since the Bold's Basal growth medium is a well-known and used growth medium for optimum

Chlorella spp. and other green algae growth. The use of the Box-Behnken design allowed for prediction of results between the three levels, therefore further concentrations and time lengths were unnecessary.

3.5.1 Growth of inoculation culture for design of experiments

The growth of the inoculation culture was followed tentatively by the cell count of the mixture, Figure 3:4. The technique for collecting data for growth curves of algae required two main provisos: (i) that there may have been agglomeration of cells within the culture which could have led to false readings, either higher or lower than the average value; and (ii) that in different stages of cell growth there could have been widely varying cell populations due to cell division or stage of cell mitosis. Prior to cell division there were less cells present in the growth medium, but the cells present were very large; immediately subsequent to cell division there were many tiny cells, which over time grew much larger to repeat the cell division cycle (mitosis) – hence the exponential growth seen from day 3 – 8. In Figure 3:4, the number of cells followed a general trend of increasing until day 8, on day 9 the growth culture had dramatically started to agglomerate which continued until visually on day 11 there were no algal cells remaining in the growth medium. Figure 3:4 shows the inoculation culture cell count which continued to show healthy proliferation of cells (until day 8). The inoculation culture for the Box-Behnken tests was removed on day 4 of growth.

The samples removed on day 4 were grown up in 24 different culture environments, with 3 control samples, and after varying time intervals were harvested, dried, the lipids extracted and transesterified using sulfuric acid. (See sections 4.2, 4.3, 4.6 and 4.12). The resultant FAMEs were then analysed by GCMS and analysed by the Box-Behnken statistical method. General trends were observed which affected repeating experiments, due to the biological complexity of algae growth; differences were noted in preliminary control samples. This biological complexity was observed when cultures were grown up at different times from the same parent culture in identical conditions gave varying results, this lead to unreliable data sets; therefore this study was carried out on a smaller but more consistent scale than first planned. The use of algae from different parent cultures or the same parent cultures at different times gave the same overall trends, but varying specific lipid masses.

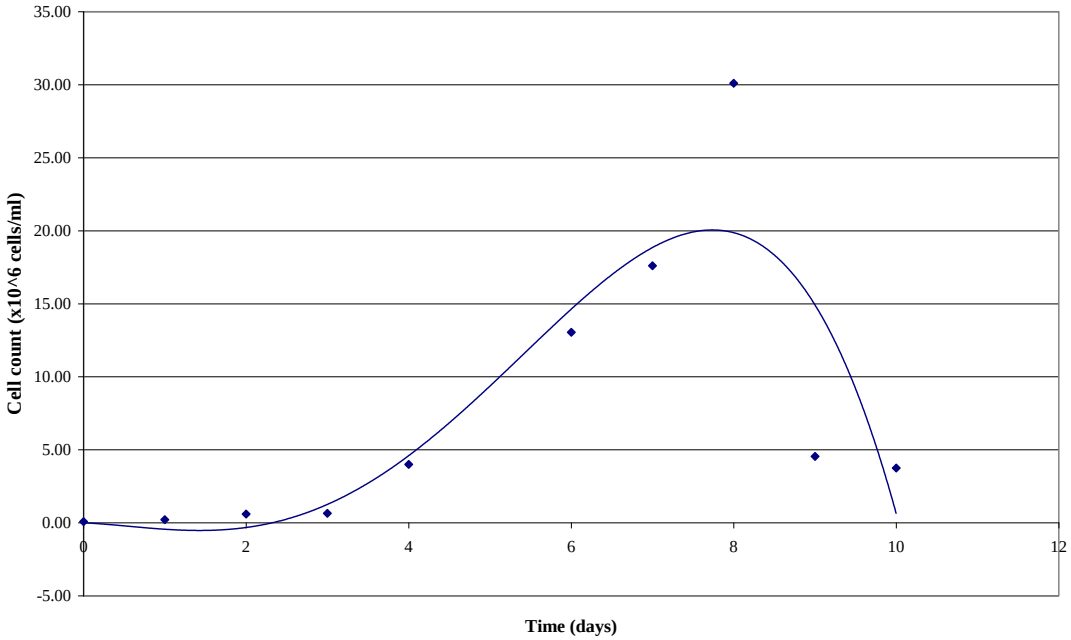

Figure 3:4 – Growth curve for Box-Behnken *Chlorella emersonii* inoculation culture; grown under standard conditions of white continuous light, 25 ^{o}C, Bold's Basal solution and 3 % carbon dioxide (Table 12)

Nitrate, phosphates, iron concentrations and culture time were chosen to be varied for the Box-Behnken tests due to differences in their reported critical requirement for *Chlorella emersonii* growth leading to changes in lipid amounts and FAME profiles.[17]

For each of the analysed parameters the percentage of the FAME constituent being looked at as a total of all FAMEs yielded as well as the actual mass of the constituent obtained in nanogrammes as the overall amount of FAME obtained is dependent upon the growth conditions.

3.5.2 Effect of nutrients and time on oleic acid methyl ester (C18(1)) synthesis

The analysis of growth parameters on the percentage of oleic acid produced by the algae was measured as oleic acid methyl ester (C18(1)) produced from the transesterification of the algal lipid. C18(1) analysis was carried out due to its superior qualities as a biodiesel fuel component for automobiles such as its cold flow properties, oxidative stability and ignition quality.

The percentage of lipid produced by the algae which was C18(1) under different conditions is shown in Figure 3:5 whilst the overall amount of oleic acid in nanogrammes produced by the algae is shown in Figure 3:6.

Figure 3:5a shows that the amount of oleic acid produced by the algae cells under increased nitrate conditions was a general trend regardless of how much iron sulphate there is. Whilst at low nitrate levels the concentration of iron sulphate in the growth media had a marked effect on oleic acid production; at lower iron sulphate levels there is an increase of oleic acid production when nitrates were also limited. In Figure 3:6a the highest amount of oleic acid was shown to be produced under Bold's Basal conditions, that is average iron and nitrate concentrations. At increased nitrate levels (0.375 g L^{-1}) with average, 4.98 mg L^{-1}, iron sulphate there was no significant difference between the C18(1) produced than with 0.250 g L^{-1} sodium nitrate.

Oleic acid production at high and low nitrate concentration as a percentage of the lipid produced was increased for *Chlorella emersonii* irrespective of changing phosphate concentration as seen in Figure 3:5b; compared to the normal levels of nitrates for Bold's Basal growth medium. However much more lipid (almost 60 mg) is predicted to be produced by the algae at high nitrate and high phosphate concentrations than under low nitrate and high phosphate conditions (less than 20 mg), see Figure 3:6b. The error graphs (see appendix) for Figure 3:5b and Figure 3:6b highlight that at the central points of the figures the predictive capabilities of the graphs are most accurate and these graphs should be used to predict the general trends with greater accuracy.

Oleic acid production, as a percentage of total lipid, increased with increased time at low nitrate concentrations, whilst increased growth time led to a decrease in production percentage of oleic acid at high nitrate concentration (Figure 3:5c). At high nitrate concentration there was initially comparable oleic acid production to low nitrate level growth after 7 days, around 50 %. It appears that oleic acid production is stable and readily-formed under even stressed growth conditions. This highlights that it can be preferable to encourage maximal growth of specific FAMEs by providing an excess availability of particular nutrients to algae cells over limited nutrient conditions which, despite yielding higher lipid percentages in algal cells, reduce overall growth. However,

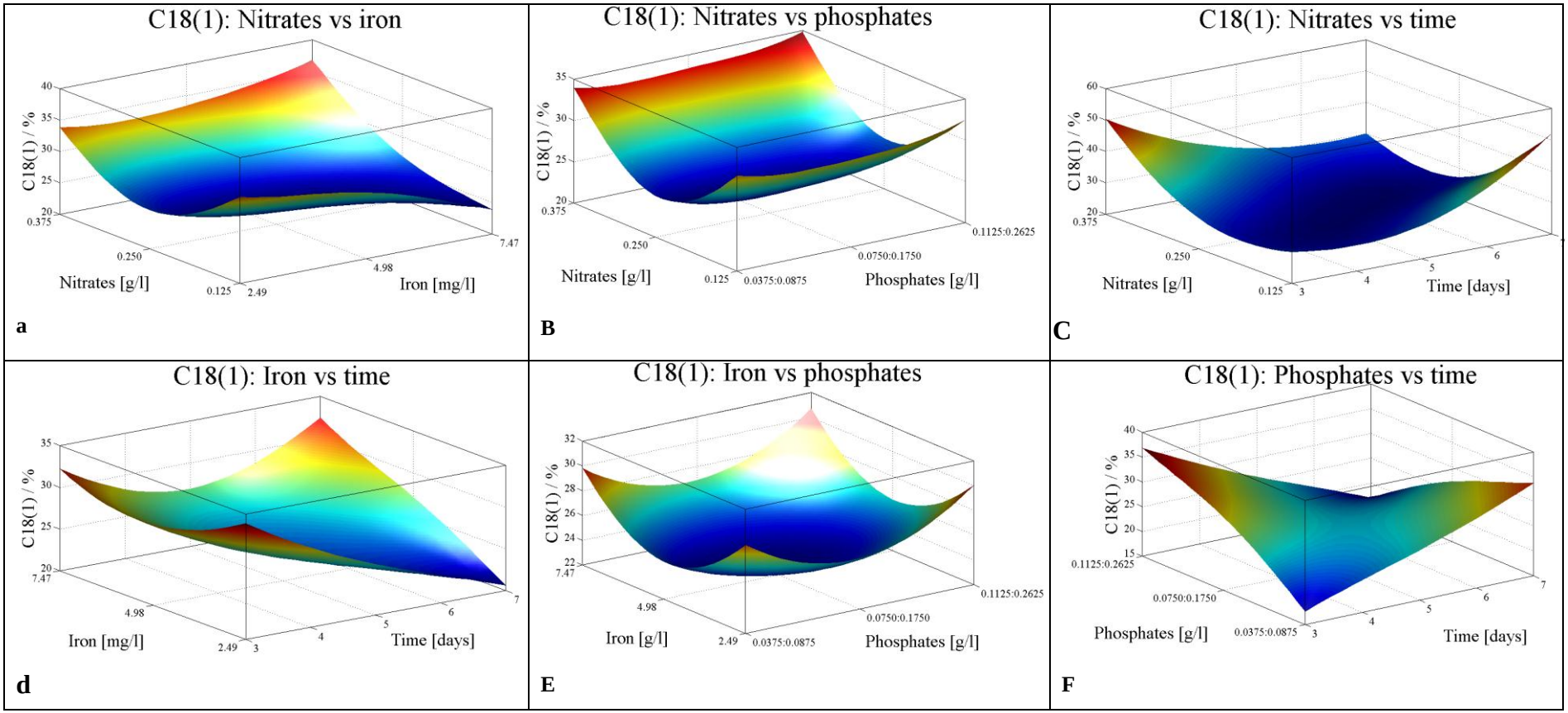

Figure 3:5 – Response surface plots of C18(1): a - nitrates vs iron concentration; b – nitrates vs phosphates concentration; c – nitrates concentration vs time; d – iron concentration vs time; e - iron vs phosphates concentration; f - phosphates concentration vs time produced by *MATLAB*® (Tables 13 – 25, 85 – 87 include error graphs, observed and calculated data points)

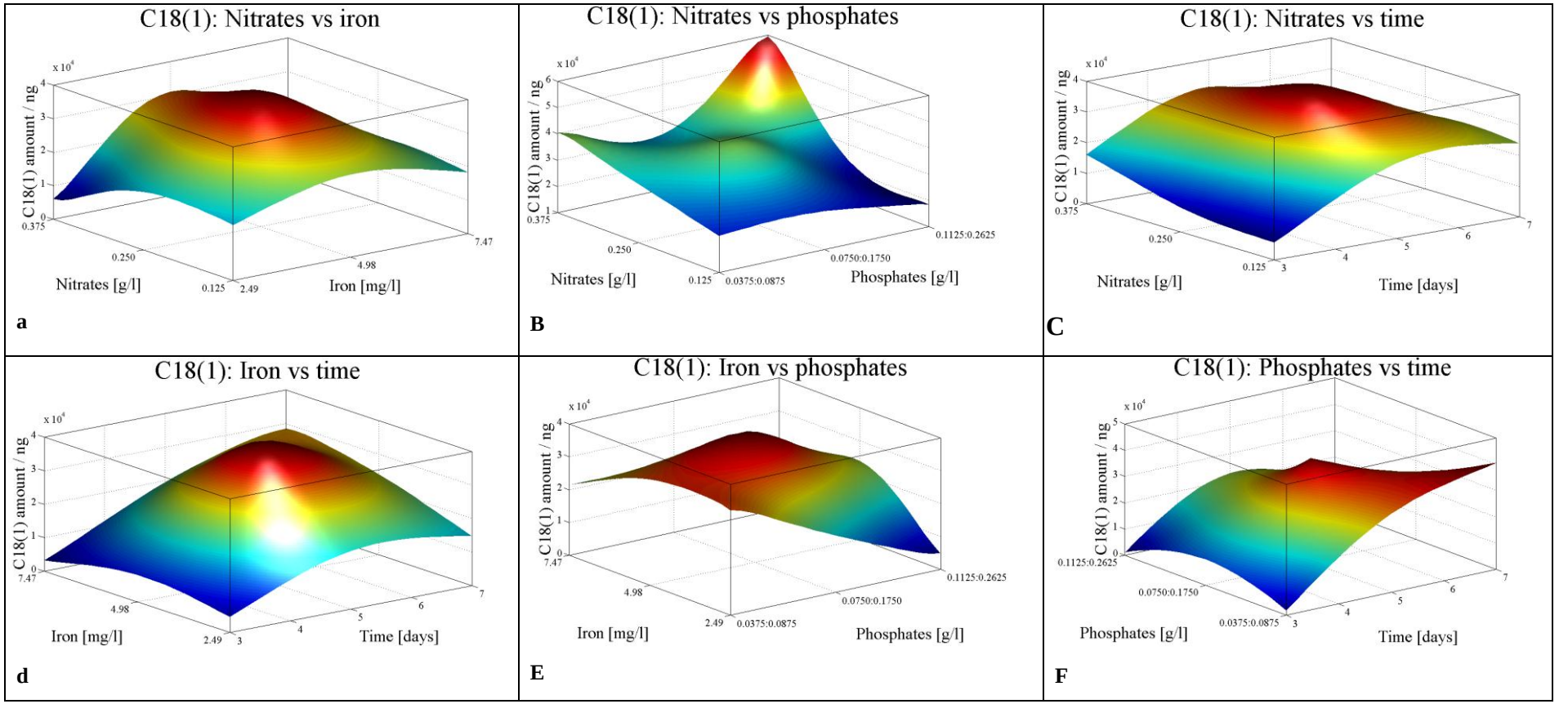

Figure 3:6 – Response surface plots of mass of C18(1) produced: a - nitrates vs iron concentration; b – nitrates vs phosphates concentration; c – nitrates concentration vs time; d – iron concentration vs time; e - iron vs phosphates concentration; f - phosphates concentration vs time produced by *MATLAB*® (Tables 13 – 25, 85 – 87 include error graphs, observed and calculated data points)

the real amounts of C18(1) detected appeared to decrease after day 6 of culture (Figure 3:6c).

Figure 3:5d displays the oleic acid production for *Chlorella emersonii* grown in Bold's Basal medium with varying trace iron sulphate concentration and analysed after different time periods. After 7 days at increased iron sulphate levels there was less C18(1) produced as a proportion of total FAMEs, than at lower levels of iron sulphate (21 % *cf.* 34 %). Whilst after three days there was little difference in the amount of oleic acid methyl ester formed regardless of the amount of iron sulphate available to the algae which could be attributed to the iron concentration not yet being limited in availability. In Figure 3:6d maximal C18(1) is observed at 4.98 mg L^{-1} iron sulphate between 5 and 6 days of culture.

In Figure 3:5e the oleic acid levels are shown to slightly increase at low and high iron sulphate concentrations when the co-factor was low or high phosphate levels, however the variance is not large (~7 %). However in Figure 3:6e the actual amount of oleic acid produced was highest at Bold's Basal levels of phosphate and slightly increased iron sulphate concentrations. Where there was reduced availability of phosphate and the trace element, iron, C18(1) production was reduced by almost 5 times; indicating that there was inadequate nutrition for formation of the oleic acid and other chain lengths and levels of saturation were preferable.

The percentage of oleic acid (Figure 3:5f) in the algal lipids was high at 3 days of culture (37 %) with high phosphate concentration whilst distinctively lower (16 %) at reduced phosphate concentration (K_2HPO_4:KH_2PO_4 – 0.-375:0.0875 g L^{-1}). After 7 days trend was reversed, however in real amounts the oleic acid amounts were drastically higher after 7 days at low phosphate concentration (Figure 3:6f).

From Figure 3:5a – c and Figure 3:6a – c it can be observed that for increased proportion of FAMEs to be C18(1) from a *Chlorella emersonii* culture an increase in nitrate concentration was advantageous, this has been further investigated in section 0. An increase in iron sulphate concentration can also positively affect the C18(1) percentage of FAME in this study (Figure 3:5a,d,e). At regular Bold's Basal solution levels of iron sulphate there was a reduction in the proportion of oleic acid methyl ester synthesised however this was counteracted by the increase in overall lipid production (Figure 3:6a,d,e).

Phosphate concentration alone did not appear to have a large effect on the percentage of oleic acid produced by the algae (Figure 3:5b,e,f), in fact it appeared to have a negative effect on oleic acid overall production (Figure 3:6e – f) unless it was abundant in combination with sodium nitrate (Figure 3:6b).

In conclusion to obtain maximal C18(1) amounts from *Chlorella emersonii* increasing nitrate and potentially phosphate concentration, along with a culture time of around 6 days in this case, would be ideal. Optimal length of culture depends more upon the size of the culture and light patterns, unless nutrients are depleted too rapidly and become limiting.

3.5.3 Effect of nutrients and time on C16(4) synthesis

C16(4) synthesis was studied more closely than other FAMEs since it is not formed in terrestrial crops from the transesterification of lipids; as mentioned in section 3.1. C16(4) is potentially formed due to the simplistic nature of algal growth and as such there is a more stable environment for the highly unsaturated lipid to form. Such a high degree of unsaturation leads to interesting properties in the biodiesel blend, even when it is present in proportions of less than 5 %. There is an increase in cold flow properties due to a decrease in viscosity which is advantageous in colder climates, however due to the higher degree of unsaturation the C16(4) FAMEs are susceptible to faster degradation due to the increased number of double bonds, particularly at higher temperatures such as found in the engine. As reported in section 1.2.2 simulated accelerated degradation of *Chlorella emersonii* biodiesel, grown under standard conditions, was more than 10 times slower than that of rapeseed biodiesel samples provided by *British Petroleum*, due to natural lipid soluble anti-oxidants.

An increase in nitrate concentration generally increased the amount of C16(4) as part of the triglycerides produced by the *Chlorella emersonii* particularly at reduced phosphate concentration and average iron sulphate (Figure 3:8a – c). An increase in percentage of C16(4) FAME was observed with a decrease in phosphates levels particularly at higher nitrate levels (Figure 3:7b) this was reflected in the overall amount of C16(4) (Figure 3:8b). Reduced phosphate concentration also yielded more C16(4) when combined with increased iron sulphate concentration and increased culture time (Figure 3:7e – f and Figure 3:8e – f). With decreased iron levels and longer growth times the C16(4) production increased to the

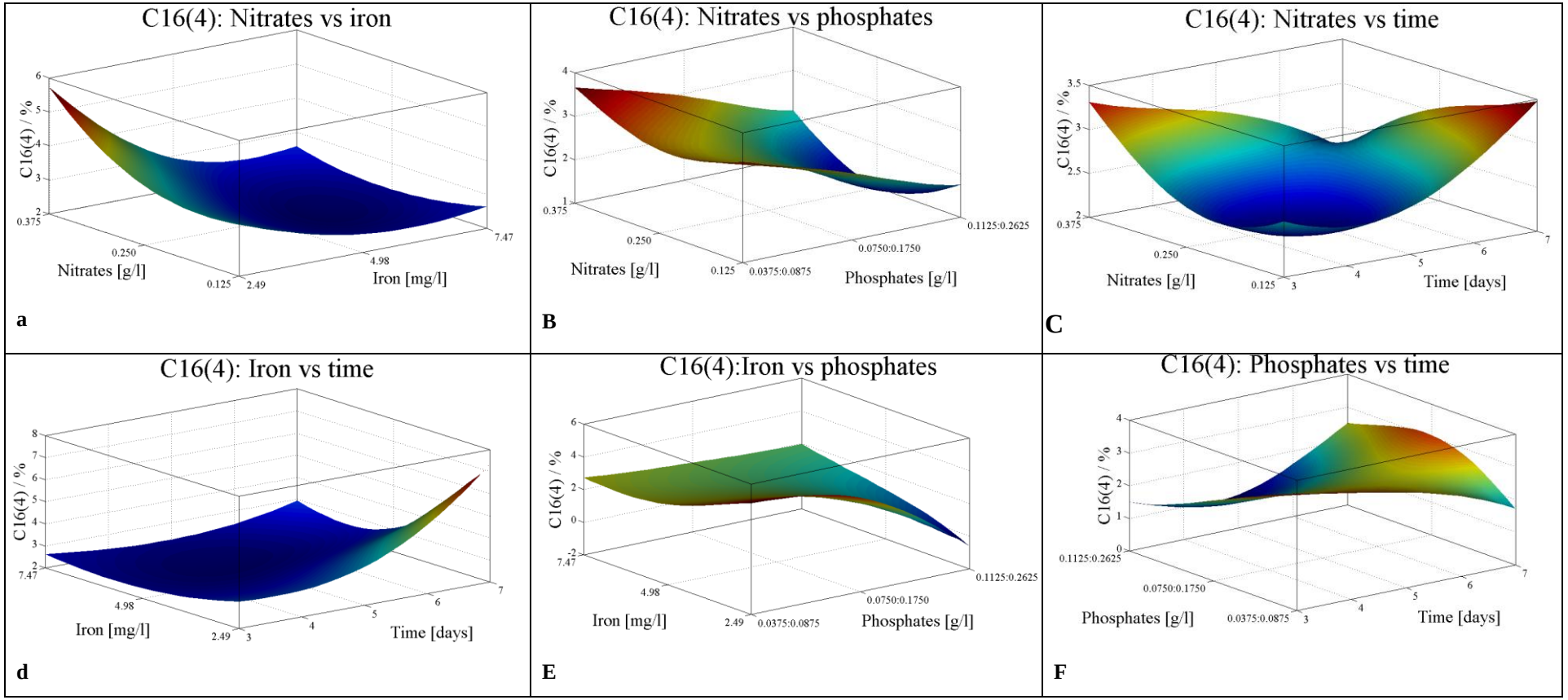

Figure 3:7 – Response surface plots of C16(4): a - nitrates vs iron concentration; b – nitrates vs phosphates concentration; c – nitrates concentration vs time; d – iron concentration vs time; e - iron vs phosphates concentration; f - phosphates concentration vs time produced by *MATLAB*® (Tables 26 – 38, 85 – 87 include error graphs, observed and calculated data points)

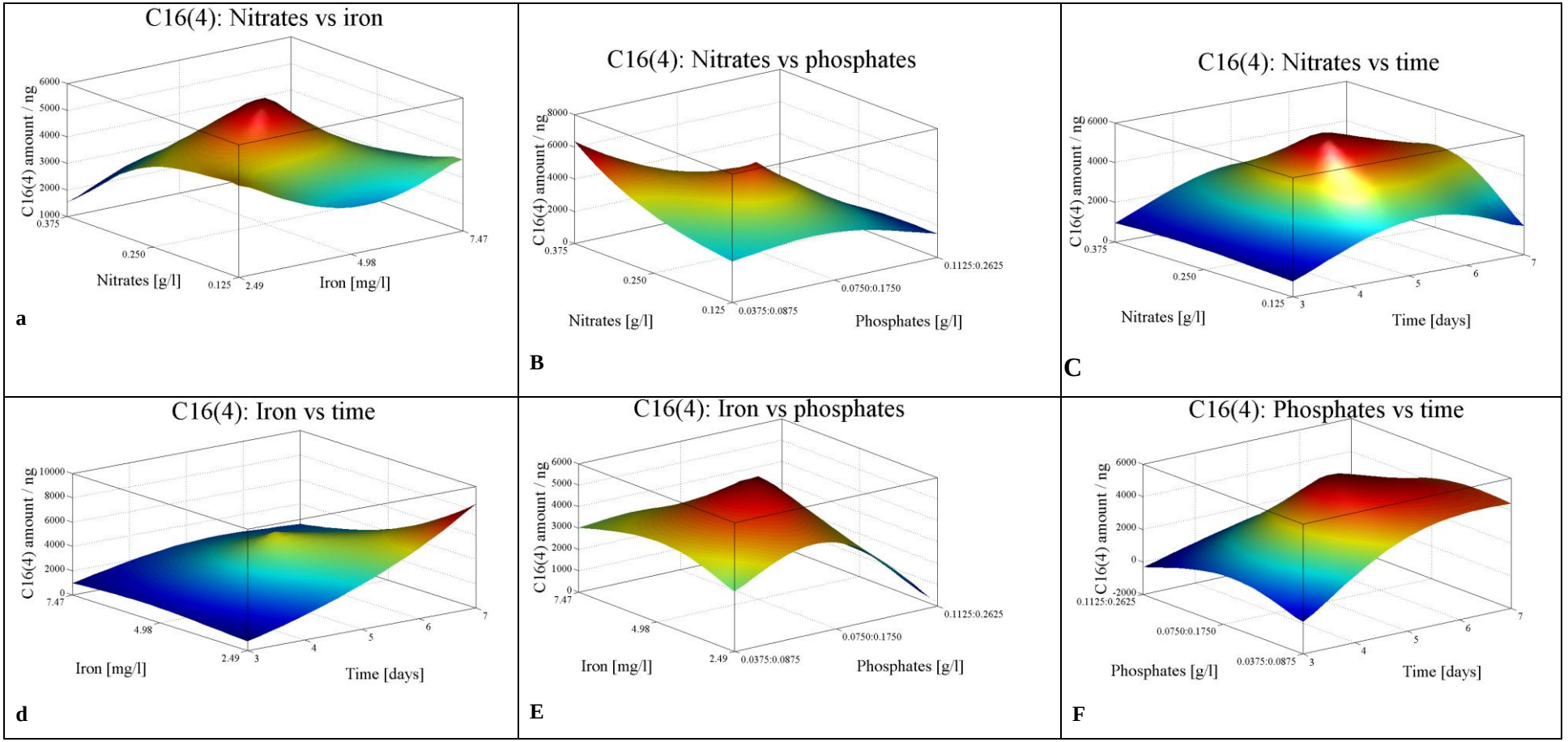

Figure 3:8 – Response surface plots of mass of C16(4) produced: a - nitrates vs iron concentration; b – nitrates vs phosphates concentration; c – nitrates concentration vs time; d – iron concentration vs time; e - iron vs phosphates concentration; f - phosphates concentration vs time produced by *MATLAB*® (Tables 26 – 38, 85 – 87 include error graphs, observed and calculated data points)

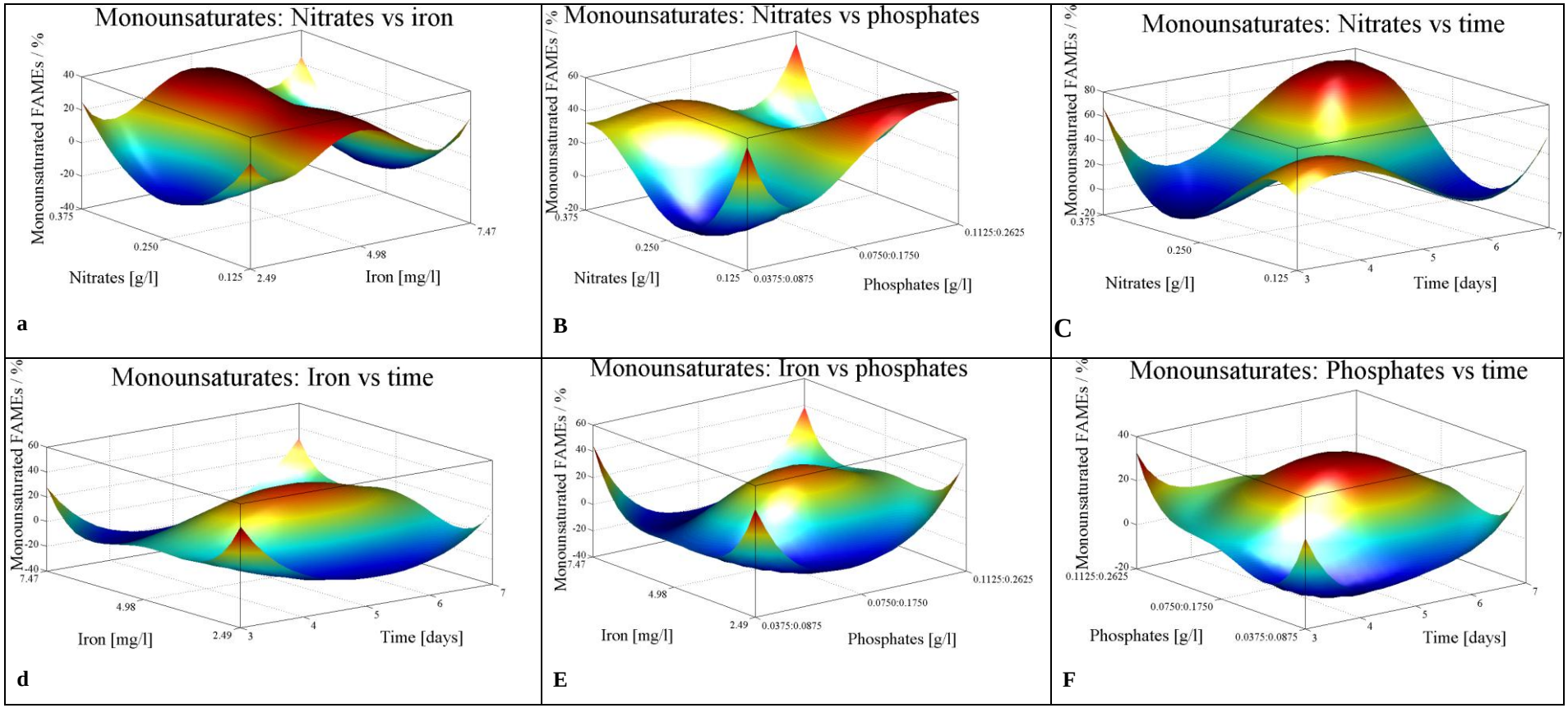

Figure 3:9 – Response surface plots of monounsaturates: a - nitrates vs iron concentration; b – nitrates vs phosphates concentration; c – nitrates concentration vs time; d – iron concentration vs time; e - iron vs phosphates concentration; f - phosphates concentration vs time produced by *MATLAB*® (Tables 39 – 51, 85 – 87 include error graphs, observed and calculated data points)

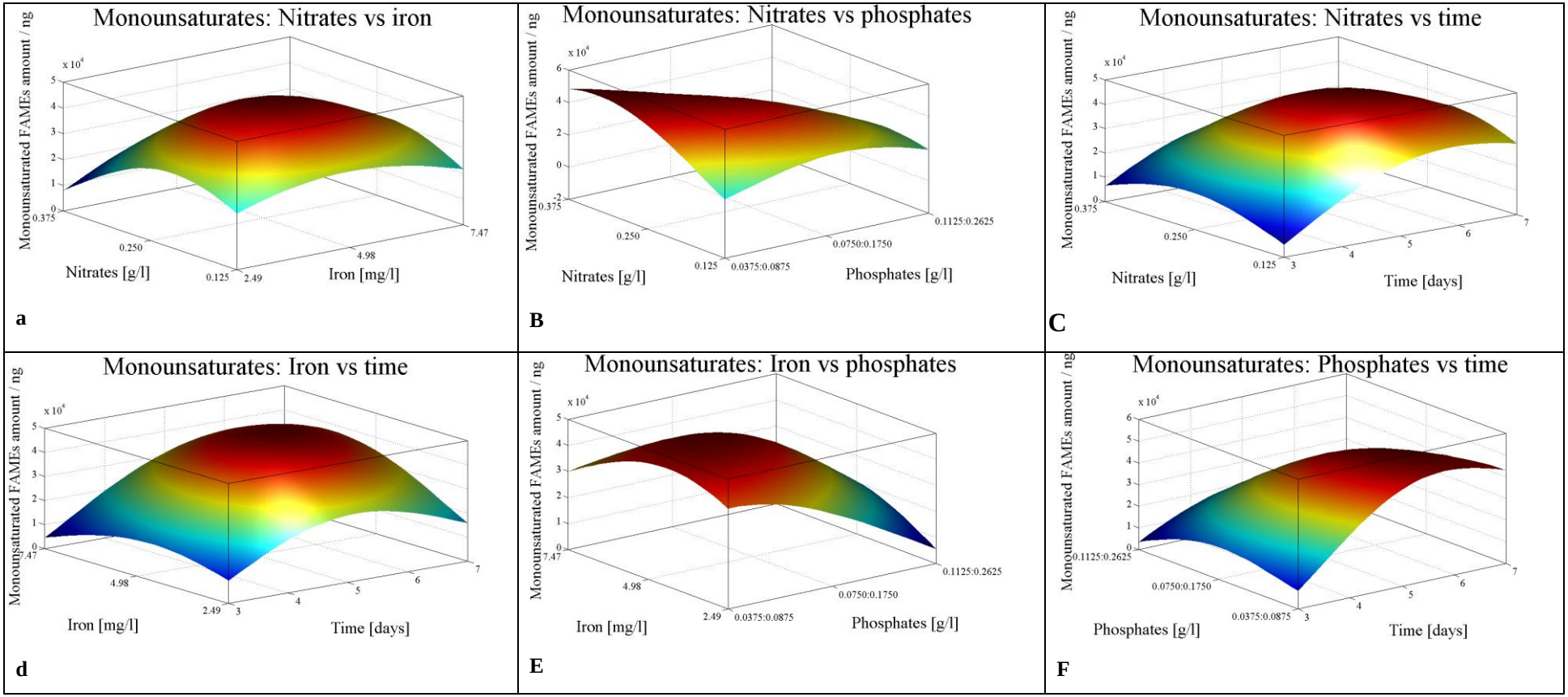

Figure 3:10 – Response surface plots of mass of monounsaturates produced: a - nitrates vs iron concentration; b – nitrates vs phosphates concentration; c – nitrates concentration vs time; d – iron concentration vs time; e - iron vs phosphates concentration; f - phosphates concentration vs time produced by *MATLAB*® (Tables 39 – 51, 85 – 87 include error graphs, observed and calculated data points)

highest seen for *Chlorella emersonii,* in this study, of almost 8 % (Figure 3:7d) and 8.5 mg (Figure 3:8d).

Over an increased cultivation time, there was an increase in proportion of C16(4) yielded and also generally an increase in C16(4) amount (Figure 3:7c,d,f and Figure 3:8c,d,f) for *Chlorella emersonii*.

3.5.4 Effect of nutrients and time on monounsaturated fatty acid methyl ester production

The individually identified FAMEs were then categorised into three groups according to the degree of unsaturation with the fatty acid methyl ester chain; either no unsaturation (saturated), one degree of unsaturation (monounsaturated) and more than one degree of unsaturation (polyunsaturated). Monounsaturated FAMEs are usually the most desirable for use as biodiesel in automobiles due to the properties described in section 3.5.2. The culture conditions which yield the highest percentage of monounsaturated FAMEs are perhaps those which are most desirable for biodiesel production in this study.

Generally higher percentages of monounsaturates are yielded most reliably from *Chlorella emersonii* cultivated with regular Bold's Basal solution conditions or conditions with slightly increased nitrate levels (Figure 3:9a – f). This slight increase in the biodiesel desirable monounsaturates led to the use of increased nitrates in the time observed FAME profiling in section 0. The highest percentage of monounsaturates, ~70 %, is obtained at around 6 days of culture for slightly increased nitrate concentration (Figure 3:9c).

For the actual amounts of monounsaturated FAME , the highest mass was produced at just higher than Bold's Basal solution levels of sodium nitrate, this may be due to increased protein and nucleic acid production allowing for faster proliferation of algal cells (Figure 3:10a – c) under these conditions. Whilst a reduction in overall lipid amount may have occurred, this was usually more than compensated for by the increase in overall algal biomass produced. This was corroborated by the increase in monounsaturates at increased time of culture (until culture death occurs - Figure 3:10c,d,f). For the trace element iron, Bold's Basal concentrations were sufficient for increased monounsaturated accumulation (Figure 3:10a,d,e). Reduced or average phosphate concentration appeared to give the

highest amounts if monounsaturated FAME from transesterified algal lipids (Figure 3:10b,e,f).

3.5.5 Effect of nutrients and time on polyunsaturated fatty acid methyl ester production

Polyunsaturated FAMEs could be of particular importance especially for jet fuel if issues with degradation can be proven to be overcome. Polyunsaturated FAMEs are the most easily oxidised FAMEs, yet have been established not to break down when processed with other lipid soluble compounds from the algae. The antioxidants, such as α-tocopherol, are extracted with the lipids from the algae and therefore oxidation is not deemed to be an issue for these polyunsaturates as it would be in other biodiesel types (see section 3.5.3). Alternatively reliable, affordable anti-oxidants can be added to the blend of FAMEs (such as those currently added by the fuel industry to fossil-derived liquid fuels). The interesting properties of highly polyunsaturated FAMEs have been largely unrealised due to their instability however they could prove superior to fossil fuel alternatives if this can be overcome due to their improved cold properties and lubrication quality.

Generally increased nitrate levels led to an increase in proportion of polyunsaturated triglycerides formed by the *Chlorella emersonii* (Figure 3:11a – c). Up to 45 % of the total FAMEs were made up of polyunsaturates at high nitrate concentrations and low iron concentrations, which was reasonably high compared to other biodiesel sources. This increase was reflected in the actual amount of polyunsaturates yielded, however the effect was not synergetic with high phosphates (Figure 3:12a – c). For iron sulphate concentrations which were higher or lower than that in Bold's Basal solution there was increased polyunsaturate formation over time as well as increased polyunsaturates at high phosphate levels (Figure 3:11d – e). Iron sulphate abundance was not required for increased proportions of polyunsaturates when nitrate was in excess (Figure 3:11a). However, for overall maximised amounts of polyunsaturates Bold's Basal solution concentrations of iron sulphate (4.98 mg L^{-1}) were best, this may have been due to the instability of polyunsaturates to oxidation at extreme levels of iron sulphate (Figure 3:12a,d,e). Generally an increase in phosphate concentration led to a reduction in polyunsaturated FAMEs in the resultant biodiesel mixture (Figure 3:12b,e,f).

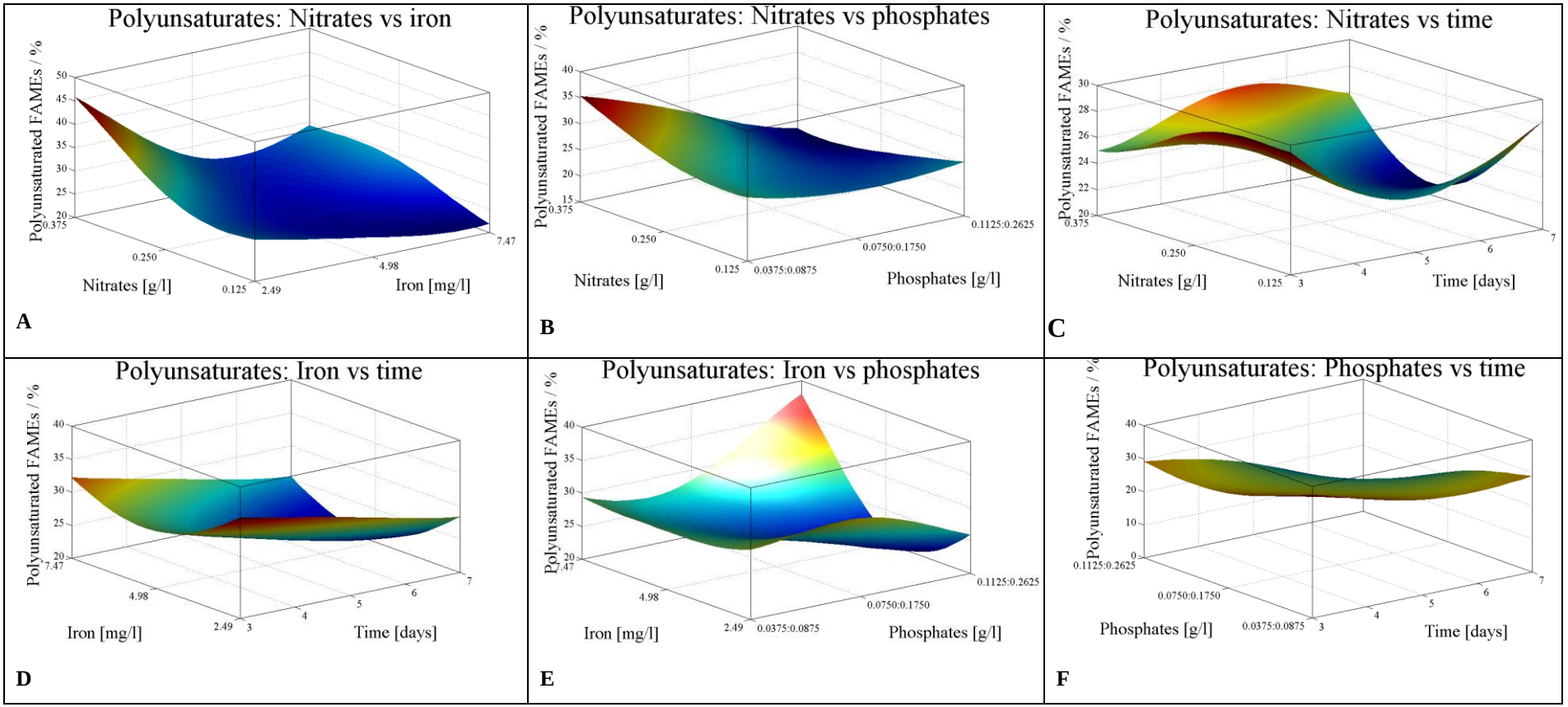

Figure 3:11 – Response surface plots of polyunsaturates: a - nitrates vs iron concentration; b – nitrates vs phosphates concentration; c – nitrates concentration vs time; d – iron concentration vs time; e - iron vs phosphates concentration; f - phosphates concentration vs time produced by *MATLAB*® (Tables 52 – 64, 85 – 87 include error graphs, observed and calculated data points)

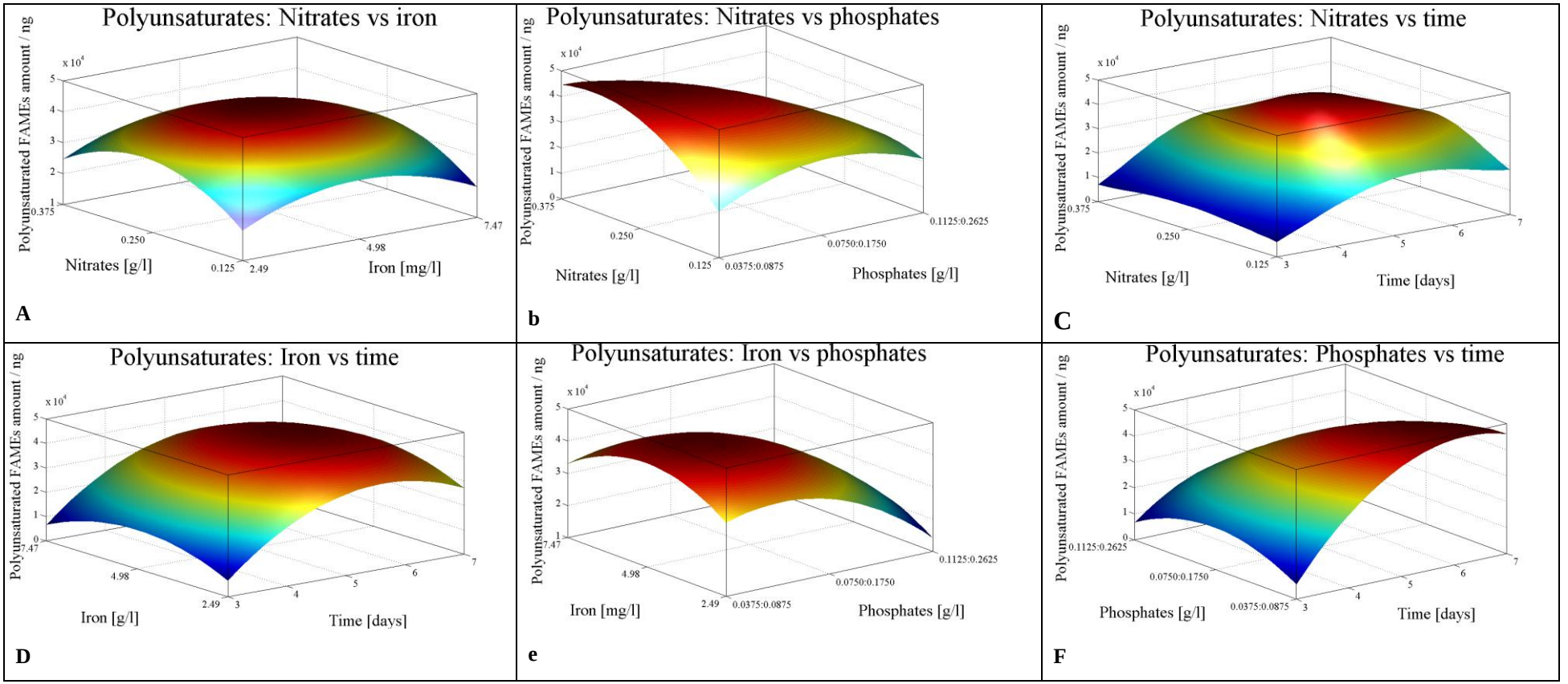

Figure 3:12 – Response surface plots of mass of polyunsaturates produced: a - nitrates vs iron concentration; b – nitrates vs phosphates concentration; c – nitrates concentration vs time; d – iron concentration vs time; e - iron vs phosphates concentration; f - phosphates concentration vs time produced by *MATLAB*® (Tables 52 – 64, 85 – 87 include error graphs, observed and calculated data points)

Increasing cultivation time reduced the polyunsaturate proportion when analysed with iron and phosphate (Figure 3:11d,f), however with nitrate the results were fluctional (Figure 3:11c). The proportion of polyunsaturated FAMEs were also maximal when algae was harvested after 3 days of growth, this suggests that the polyunsaturates are either broken down within the algae cells or the triglycerides are synthesised further perhaps into longer chain monounsaturates or saturates. The overall amounts of polyunsaturated triglyceride accumulated by the algae reached a maximum at around 6 days (Figure 3:12c,d,f). The highest proportions of polyunsaturated FAMEs were synthesised before 5 days of growth; this could be have been suggestive of denser growth cultures selecting against polyunsaturated chains on triglycerides or that bountiful nutrients were required for the formation of these highly unsaturated species.

3.5.6 Effect of nutrients and time on saturated fatty acid methyl ester production

Desirable, reliable fuels can consist of purely saturated FAMEs which are specifically blended to provide the fuel properties required. However to produce an affordable fuel from biodiesel the separation of the constituent parts into saturated and unsaturated would be prohibitively expensive. A higher proportion of the more reliable saturated FAMEs are favourable to obtain the ASTM and EN14214 credentials for biodiesel properties as there is a strict limitation on the number of unsaturates present, as measured by iodine number. The culture conditions which lead to saturated FAMEs are thus of interest for the production of dependable fuel.

An increase in the proportion of saturated lipids was seen with a reduction in nitrate concentration, particularly at high iron sulphate concentrations – <80 % (Figure 3:13a – c). For overall saturated FAME yield a cultivation solution of standard Bold's Basal solution or just higher than the 0.250 g L^{-1} concentration gave maximal output (Figure 3:14a – c). Reduced phosphate concentrations resulted in a higher proportion of saturates, particularly when combined with low nitrate concentration (Figure 3:13b) and high iron concentration (Figure 3:13e). The maximum actual amounts of saturates for varying phosphate concentrations were found at Bold's Basal solution concentrations or slightly lower than standard concentrations (Figure 3:14b,e,f), suggesting that shortage of phosphate is not an obstacle for producing triglycerides in this case.

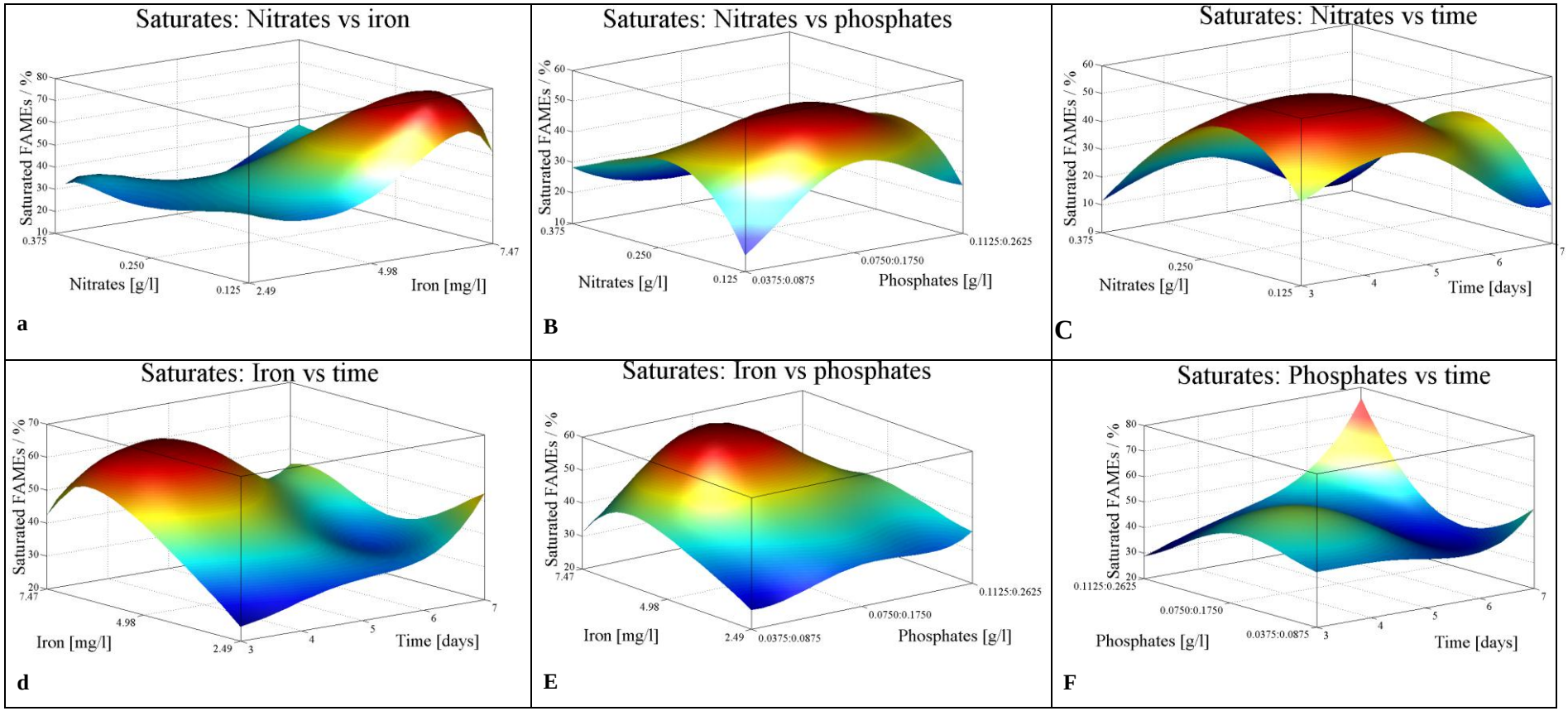

Figure 3:13 – Response surface plots of saturates: a - nitrates vs iron concentration; b – nitrates vs phosphates concentration; c – nitrates concentration vs time; d – iron concentration vs time; e - iron vs phosphates concentration; f - phosphates concentration vs time produced by *MATLAB*® (Tables 65 – 77, 85 – 87 include error graphs, observed and calculated data points)

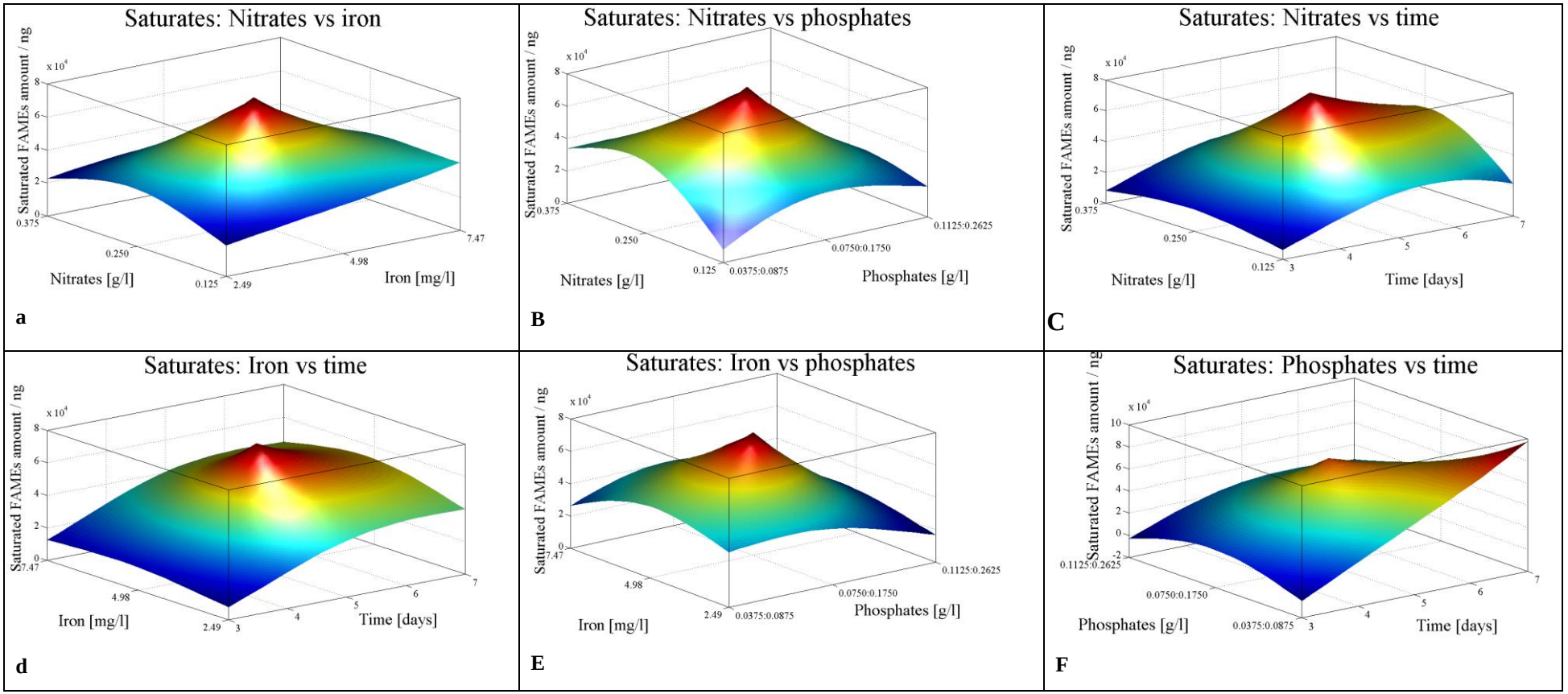

Figure 3:14 – Response surface plots of mass of saturates produced: a - nitrates vs iron concentration; b – nitrates vs phosphates concentration; c – nitrates concentration vs time; d – iron concentration vs time; e - iron vs phosphates concentration; f - phosphates concentration vs time produced by *MATLAB*® (Tables 65 – 77, 85 – 87 include error graphs, observed and calculated data points)

As for the other nutrients, the standard Bold's Basal solution concentration of iron yielded the highest saturate amounts (Figure 3:14a,d,e), whilst the proportion of saturates formed increased under higher iron sulphate levels (Figure 3:13a,d,e).

Overall a reduction in the proportion of saturated FAMEs was observed in the transesterified lipids which have been cultivated for a longer time (Figure 3:13c – d), apart from the increase noted at high phosphate concentration (Figure 3:13f). The percentage of saturated FAMEs (Figure 3:13f) increased dramatically when algae were grown for 7 days compared to 3 days at high phosphate concentration (~80 % *cf.* ~50 %). This was corroborated by the lower proportion of polyunsaturated FAMEs under the same conditions (Figure 3:11f). At lower phosphate concentration there was less difference observed – the proportion of saturated FAMEs varied between ~30% to ~40 % (Figure 3:13f). The total amount of saturates peaked at 5 – 6 days, but generally increased with increased cultivation time (Figure 3:14c,d,f).

3.6 Effect of nutrients and time on total lipid

The total amount of lipid in the *Chlorella emersonii* cultures is a combination of the composition of the cell and the overall mass of the algae biomass cultivated. The total amount of lipid obtained is important for the viability of algae, particularly *Chlorella emersonii*, as a potential source of biodiesel.

From the results obtained it appeared that the concentration of sodium nitrate for maximum lipid was the same as the Bold's Basal solution or just above that; an excess of nitrates encouraged rapid growth of algae (Figure 3:15a – c). An increase in phosphate concentration increased the lipid production of the algae, though not as much as slightly increased nitrate and phosphate concentrations (Figure 3:15b).This increase in lipid production at slightly increased nitrate and phosphate concentration suggests that wastewater could be used for successful algae cultivation if diluted.

Increased amounts of iron can be beneficial for the increase in total FAMEs produced from the algae lipids if phosphate concentration was low (Figure 3:15e). Over time the culture generally accumulated more lipid for conversion into FAMEs regardless of the culture conditions (Figure 3:15f), however when observing nitrates (Figure 3:15c) and iron (Figure 3:15d) as co-factors, 5 – 6 days produced maximal lipids in this case.

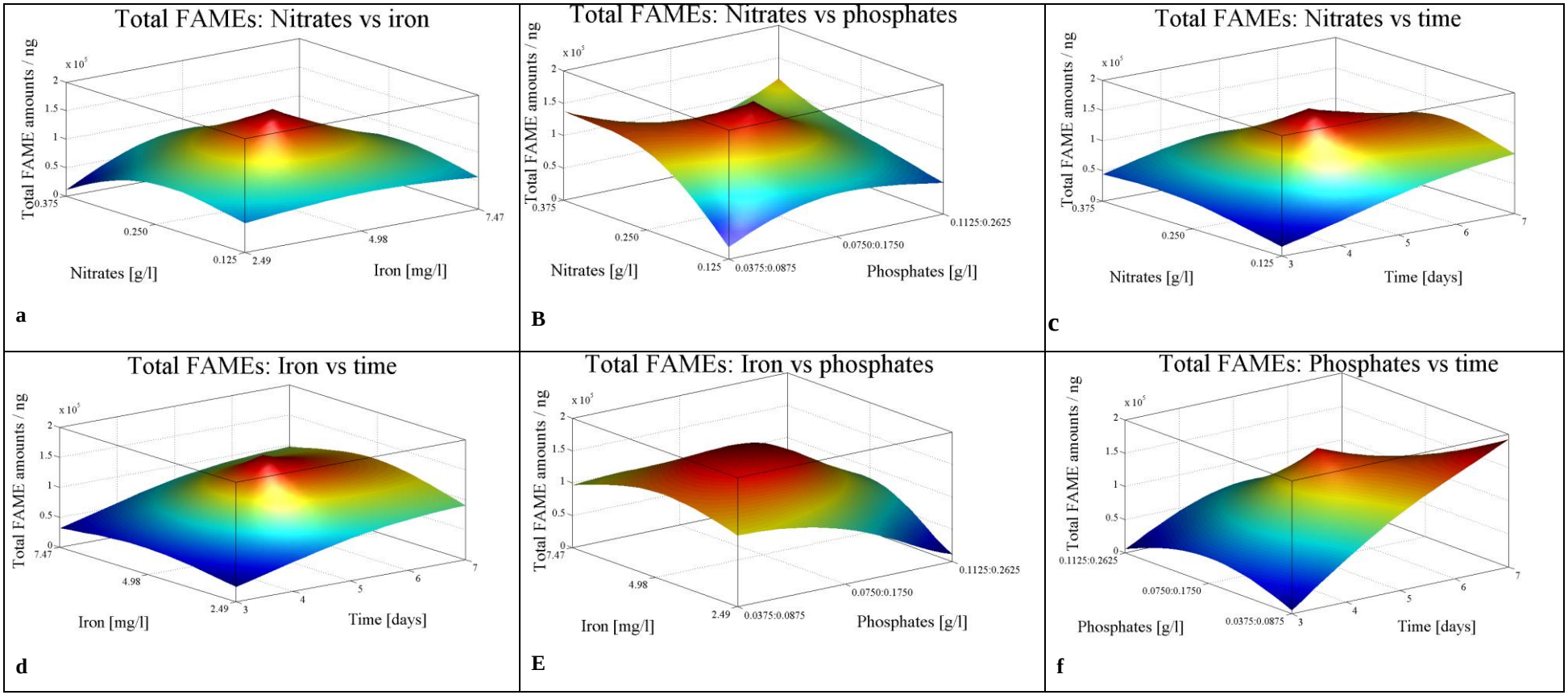

Figure 3:15 – Response surface plots of mass of total FAME produced: a - nitrates vs iron concentration; b – nitrates vs phosphates concentration; c – nitrates concentration vs time; d – iron concentration vs time; e - iron vs phosphates concentration; f - phosphates concentration vs time produced by *MATLAB*® (Tables 78 – 87 include error graphs, observed and calculated data points)

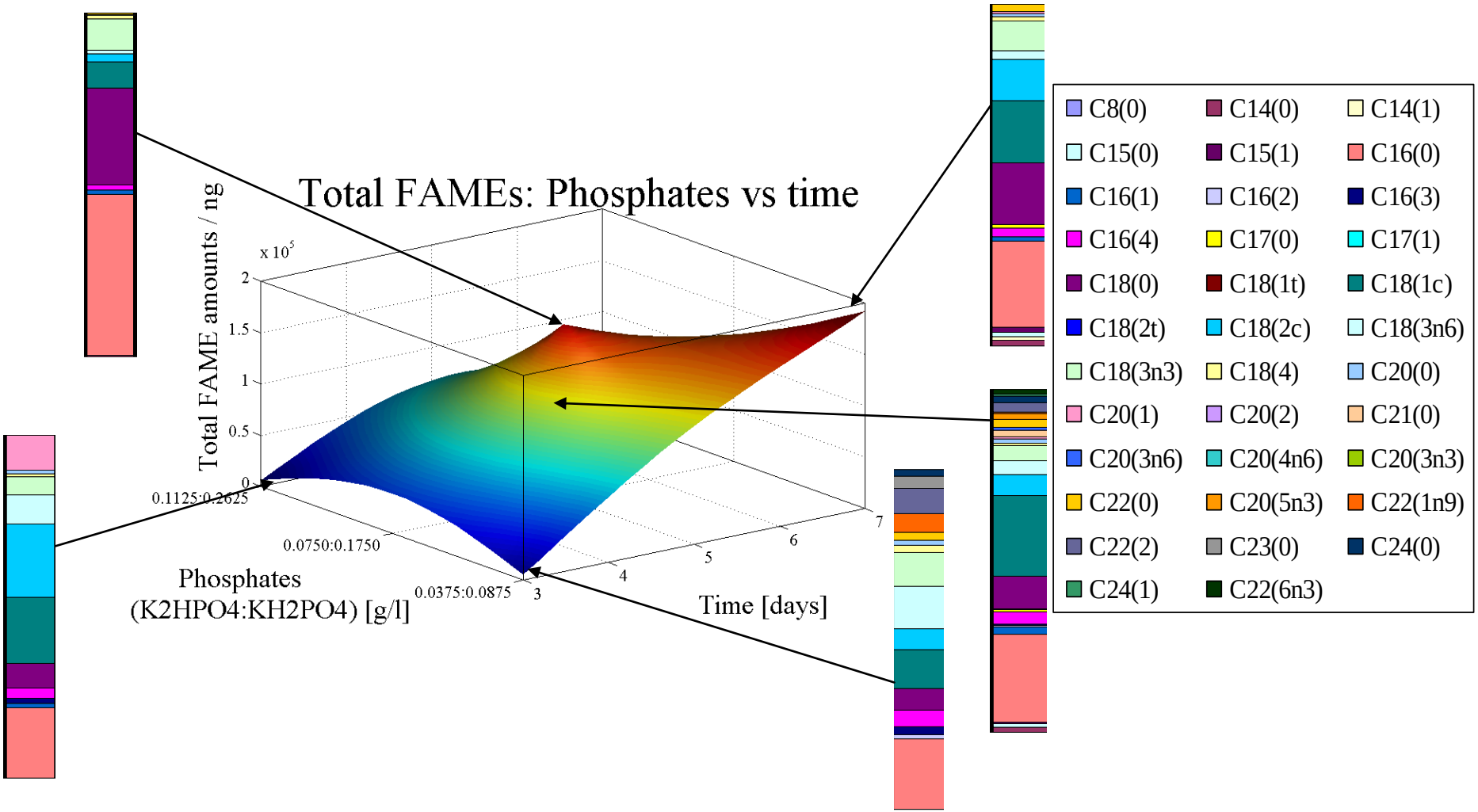

Figure 3:16 – Response surface plots of total amount of FAME produced from *Chlorella emersonii* lipids with respect to phosphate concentrations and time of cultivation; additional FAME profiles for the algal derived FAMEs (Tables 83 – 87)

Overall the triglyceride composition, observed as FAME profile was not consistent over time, suggesting that different triglycerides are formed at different points in the algal cell growth cycle. Figure 3:16 highlights the change in FAME composition in the samples at different cultivation times and at different growth conditions. Generally the triglycerides are more monounsaturated later in the growth cycle, i.e. when harvested after 7 days of cultivation, whereas there are more saturated and polyunsaturated FAMEs in the biodiesel produced from algal lipids when harvested after 3 days. The algae generally produce higher amounts of monounsaturates and saturates under wastewater conditions of increased nitrates and phosphates.

The impact of longer cultivation time leads to an increase in overall biomass which in turn leads to a larger amount of lipid produced, even if the conditions were not ideal for lipid production. The amount of algae grown in each case, usually, overcame the reduction in proportion of lipids when algae was grown under more ideal growth conditions.

3.7 Effect of time on algae growth and fatty acid methyl ester profile

This section looks more closely at time as a parameter which is fundamental to cell proliferation and lipid accumulation. As time progresses it is expected that cells will replicate until saturation of the growth medium or until one or more of the required nutrients or light becomes limiting, this can be observed by the lack of peak on day 14 (Figure 3:17). In this experiment coagulation/agglomeration of the algae cells began on day 10 and there were no remaining viable cells on day 14. Time is studied further in this experiment as it was noted in the design of experiments (see sections 3.5 – 0) that cells were still in a healthy, viable state after 7 days growth.

Chlorella emersonii was grown in Bold's Basal growth medium with increased nitrates to yield the optimised biodiesel profile for automobiles – namely to maximise the increased C18(1) (oleic acid methyl ester) observed in the previous section 3.5. These results are in comparison to literature for *Chlorella minutissima* where a decrease in nitrates yields lower C18(3) whilst giving higher C18(1) output with higher overall lipid percentage per algal cell; although there is no report the actual overall amount of lipid or resultant FAME produced for direct comparison.[18] This highlights the species specificity of growth and

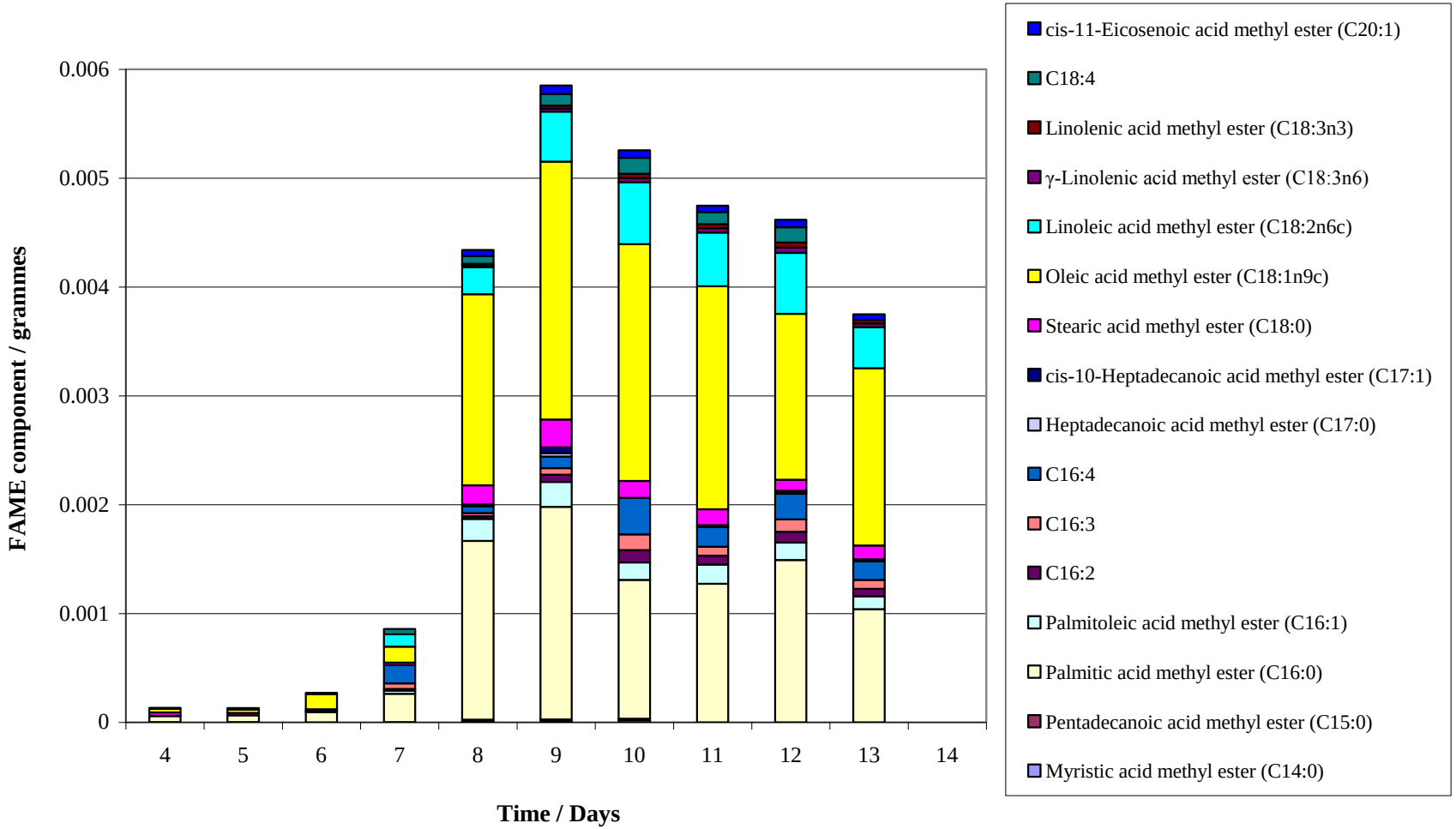

Figure 3:17 – FAME profile in grammes over time for *Chlorella emersonii* grown under increased nitrate conditions for maximised C18(1) (Table 88 - 89)

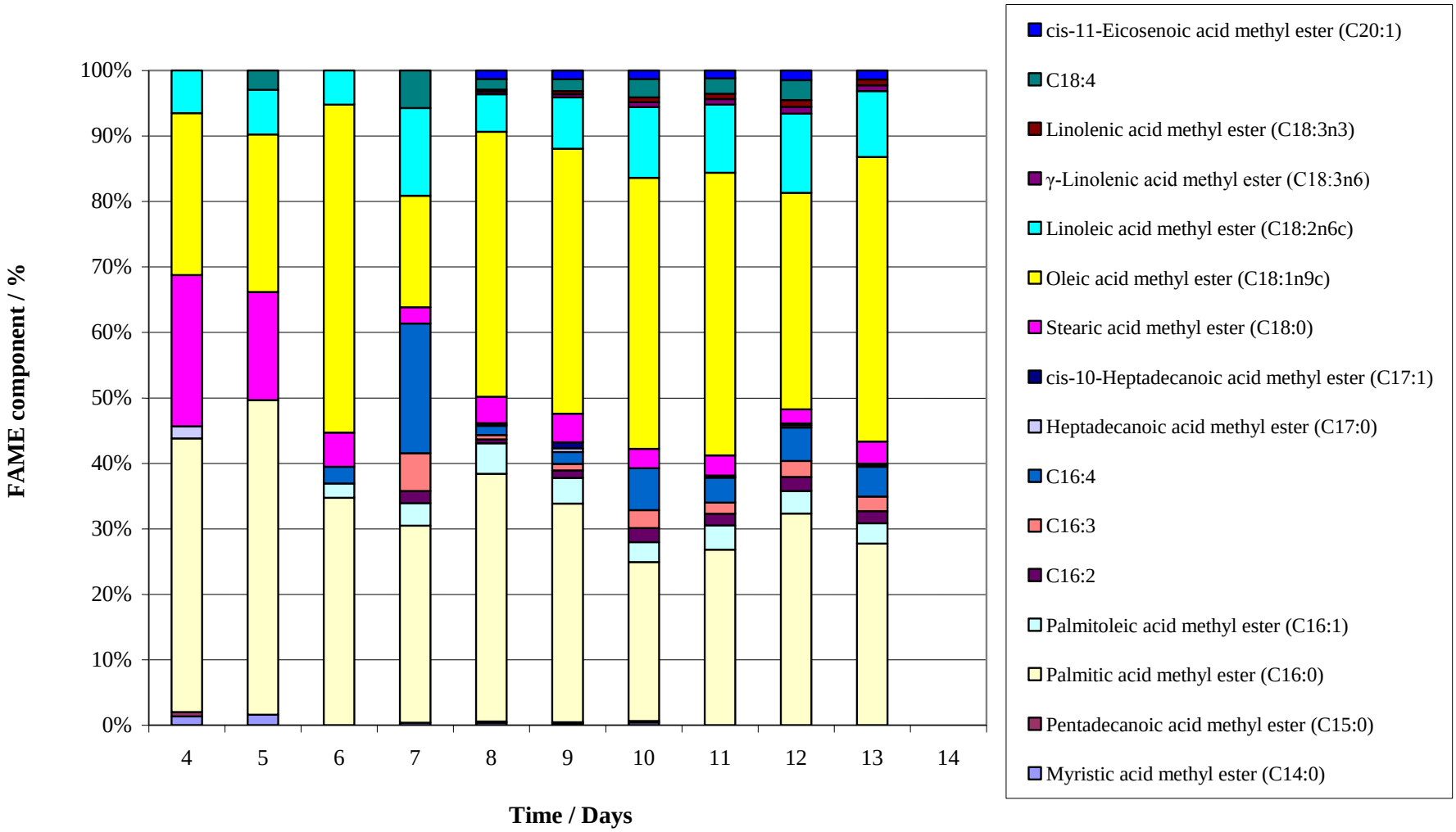

Figure 3:18 - FAME profile in percentage over time for *Chlorella emersonii* grown under increased nitrate conditions for maximised C18(1) (Table 88 - 89)

light conditions on biomass productivity and the effect on FAME profile. The data displayed in the Figure 3:17 was collated from a 100 ml sample of growth medium being harvested each day from the culture. These samples were dried, the lipids extracted and subsequently transesterified. The FAMEs obtained were then analysed by GCMS to allow for individual FAME quantification. From 6 to 7 days the overall mass of the algae per 100 ml solution doubled. Whilst on the 8th day there was a dramatic increase in yield which was matched by an increase in the proportion of C18(1) obtained. The increase in FAME amount obtained from 100 ml algae growth solution increased slightly again from day 8 – 9, but on consecutive days diminished until day 14 as mentioned above. In Figure 3:17 it can be seen that maximum lipid is obtained after 9 days. Since there is a large increase of 4.8 times of lipid amount from day 9 compared to day 7 it is more economical to cultivate algae for two extra days.

The FAME profile from day 8 onwards remained reasonably constant with mainly C16(0), C18(1) and C18(2) the three largest components and C16(4) as an important component, Figure 3:18. On day 4 and 5 C18(0) was a major component alongside C18(1) and C16(0) however this diminished on day 6 where an increase in C18(1) synthesis was observed. On day 7 where the resultant FAMEs became more varied including, C16(0), C16(4), C18(0), C18(1), C18(2) and C18(4) amongst the more notably resultant FAMEs. The growth stage of the cells may give rise to these changes in resultant FAME composition; for example the increase in C16(4) on day 7 which diminished at subsequent testing. Alternatively it may have been due to the stability of the growth culture that the amount of saturated FAMEs decreased slightly over time.

In summary, time is an important factor for algae growth with 9 days being optimal in this experiment, for maximal lipid output, regardless of the FAME components desired. The resultant FAME composition remained mostly stable after the first 7 days of growth irrespective of the coagulation of some of the cells.

3.8 Effect of carbon sources on algae growth and fatty acid methyl ester profile

A mixotrophic experiment was designed to determine whether gaseous carbon dioxide enriched air is sufficient for maximal algae growth or if further addition of carbon sources

encourage increased growth rates. This was carried out since algae utilisation of different carbon sources, as for any nutrient, is species defined.[19]

The effect of supplementary carbon sources upon algae growth and lipid accumulation was investigated using the set up shown in Figure 3:19. As can be observed by the colour of the algae in the conical flasks, the healthiest cultures (greenest) are those with no auxiliary additional carbon source, or with added ethyl acetate or glycine.

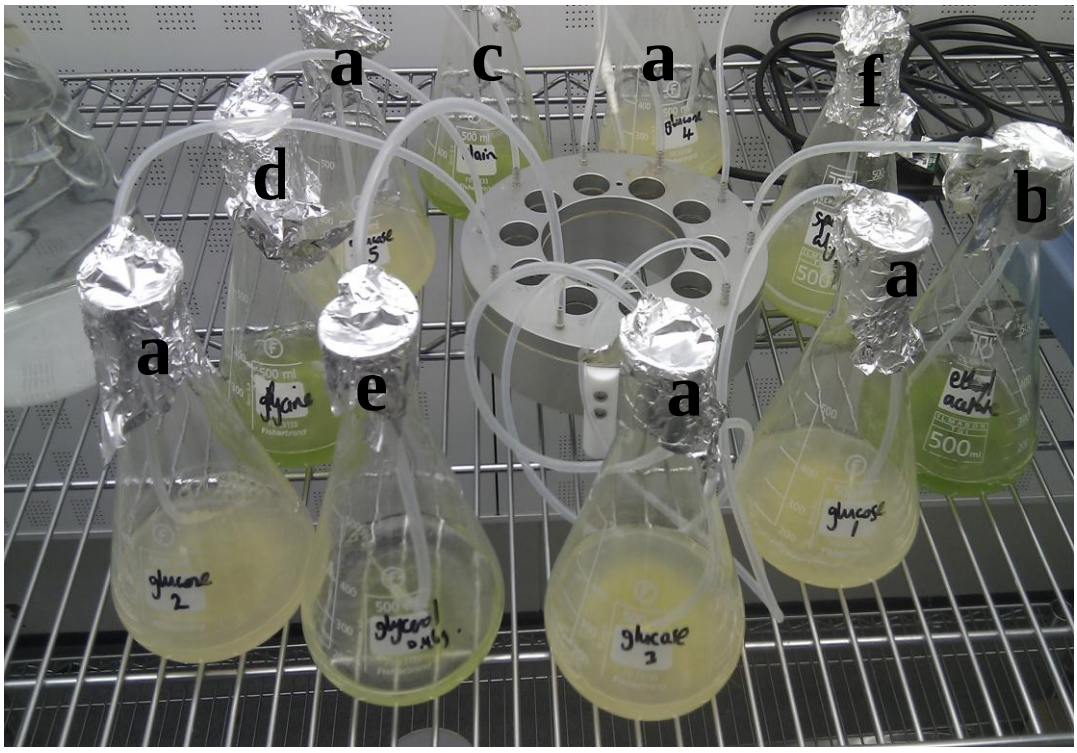

Figure 3:19 – Mixotrophic growth and set up (continuous white light, 25 ^{o}C, 3 % carbon dioxide, Bold's Basal solution): (a) additional 2 mol %/L glucose; (b) additional 2 mol %/L ethyl acetate; (c) control; (d) additional 2 mol %/L glycine; (e) additional 2 mol %/L glycerol; (f) additional 2 g spent algae

The mixotrophic growth experiments highlighted that the green algae *Chlorella emersonii* grows optimally for maximal lipid accumulation in Bold's Basal medium, with 3 % additional carbon dioxide and no other carbon source. The addition of spent algae as a potential carbon source increased the amount of C16(0) formed alongside other FAME components. This increase in C16(0) was attributed to the duplicate processing of the spent algae increasing the FAME output due to increased degradation of the cell wall releasing otherwise unprocessed lipid. This was seen as with the re-extracted spent algae there was a large increase in FAME production compared to all other tests within this experiment. This increase is due to the 2.000 g of dry spent algae having lipid extracted from it, which is

much higher than the dry weight of the algae grown up during these experiments. Due to the small scale of the experiments performed for this mixotrophic testing the samples were not freeze dried. Washing from the culture vessel was assumed to be equal for all samples, therefore allowing for comparison between sample amounts.

A 2.001 g comparison sample of dried spent algae from the same batch was re-extracted and found to contain 0.729 % lipid. There was more lipid extracted from the comparison sample (14.58 mg resultant FAME) than from the spent algae which was used as a potential feedstock for the viable algae (5.06 mg resultant FAME). This suggested that the algae do not use the spent algae as a source of carbon or any other nutrients or micronutrients. The 2.88 times reduction in the lipid amount from the re-extracted spent algae when added into the test as a feedstock suggests that the dried algae are well distributed in the growth media and is therefore removed along with supernatant. Some of the biomass was lost with the supernatant after centrifugation whilst some was unable to be adequately washed from the culture vessel. It is also possible that some of the residual lipid has been broken down under the test conditions or that the lipid leached into the supernatant over the course of the experimentation. Overall it is therefore impossible to suggest whether the addition of spent algae to fresh algae cultures encourages cell growth, has no effect or impedes algae growth.

The use of carbon sources for the manipulation of resultant FAME production can also be highlighted for example with the addition of glycine the unsaturated C16(1), C16(4), C18(2), C18(3) and C18(4) increased though overall FAME produced slightly decreased (Figure 3:20). The addition of glucose increased the percentage of monounsaturated FAMEs at the expense of saturated and polyunsaturated FAMEs, compared to no additional carbon source. The addition of spent algae drastically increased the percentage of C16(0) alongside other saturated FAMEs being produced; this had the effect of increasing the overall amount of FAME produced. The addition of ethyl acetate to the algae culture led to more than a doubling of C16(0) and C18(0) produced and a decrease in C18(3) as well as a reduction of around a third of C18(1). The addition of ethyl acetate led to a decrease in overall lipid production of around one quarter compared to algae grown with no additional carbon sources, and increased saturated FAMEs. In literature the addition of sodium acetate led to a growth stimulation compared to standard phototrophic conditions for *Chlorella vulgaris*.[20] The addition of glycerol to the growth culture yielded

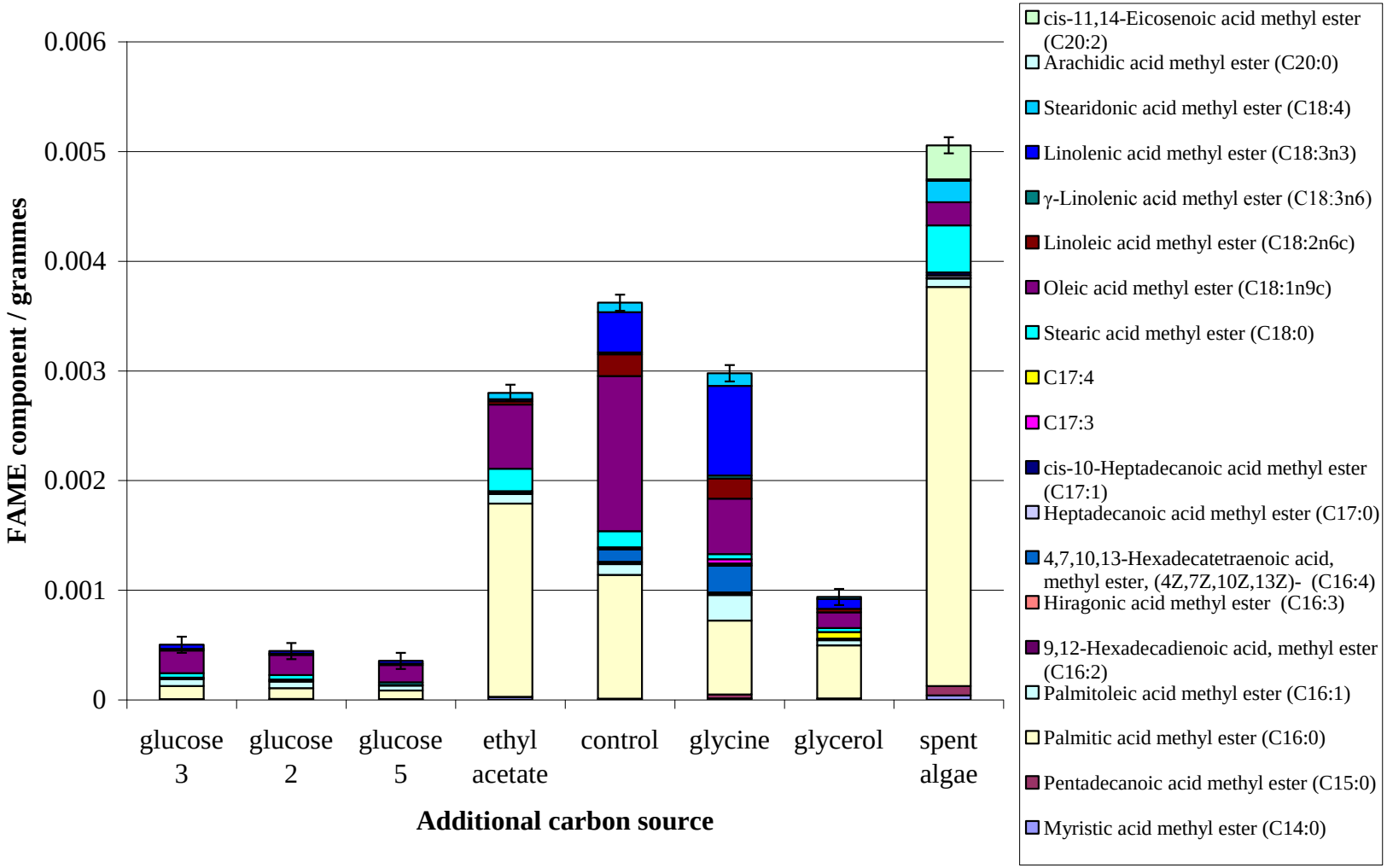

Figure 3:20 – Mixotrophic FAME composition: continous white light, 25 °C, 3 % carbon dioxide, Bold's Basal solution with: (a) additional 2 mol %/L glucose; (b) additional 2 mol %/L ethyl acetate; (c) control; (d) additional 2 mol %/L glycine; (e) additional 2 mol %/L glycerol; (f) additional 2 g spent algae (Table 90)

an increase in the saturated FAME C16(0) and also an increase in the shorter monounsaturated FAME C16(1), alongside unexpected C17(4) production. A reduction in C18(1) was observed as well as these increases, as well as an overall decrease in lipid synthesis with a co-carbon source of glycerol. This is corroborated by literature, where glycerol was found to be inhibitory to *Chlorella vulgaris* cell growth.[20]

3.9 Microwave extraction

Microwave technology for the extraction and transesterification of lipids from algae was investigated due to 14 hour and large volumes of solvent required for Soxhlet extraction method. Microwave technology requires less solvent, less energy, less time and has the potential for fewer steps than Soxhlet extraction, it also allows for automated processing of samples. This is due to the use of microwaves which with the varying electronic fields accelerates the heat and pressure inside the algae cells allowing faster and improved permeation of lipid than with mechanical or thermal methods (Section 1.4.3). These factors make microwave use potentially more environmentally and economically attractive.

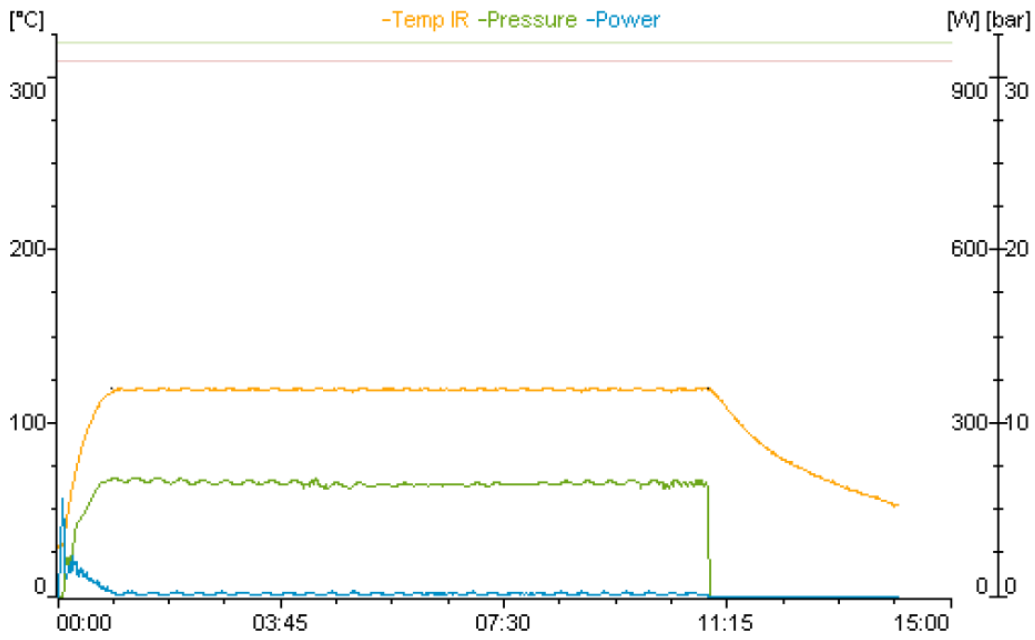

Figure 3:21 – Example of temperature, pressure and power readings throughout microwave extraction of *Chlorella emersonii* set to 120 °C for 10 minutes with chloroform: methanol (2: 1) and 2 drops of sulfuric acid

The initial lengths of time were chosen upon the recommendation from the technical supplier of the microwave who suggested that other applications for the microwave

required, generally, less than 10 minutes reaction time. It was chosen to begin at 10 minutes since the *Chlorella emersonii* algal species are known to have tough cell walls. Other green algae tested required much shorter reaction times and less harsh conditions than the *Chlorella emersonii* did. Subsequent processing with longer hold times to attempt breakdown of the algal cell walls led to degradation of the FAME molecules.

Four solvent mixes were tested, some of which simulated working with wet algae, which is more economical, since drying requires vast amounts of energy. Methanol, chloroform, chloroform: methanol (2: 1) and chloroform: methanol: water (2: 1: 1) were all tested. It is noted in Chapter 1 that if wet algae has a higher ratio of methanol added to it, this can counteract the water presence. The sole use of methanol as an alcohol was chosen as this would produce purely methylated end groups, which would allow easier analysis. Chloroform was chosen as a co-solvent to be tested, since this would aid permeation of the lipid/FAMEs through the algal cell wall.

Preliminary results from the microwave system showed algal lipid transesterification with or without the use of acid catalyst under conditions of chloroform: methanol in a 2: 1 ratio run for 15 minutes or 1 hour at 120 °C and 150 °C. The presence of water in the reaction mixture made no difference to the FAME composition observed. For all samples, regardless of their extraction-transesterification conditions the major FAME observed was C18(1) by GCMS.

When run at the higher temperature of 180 °C for 1 hour, there was a prevalence of saturated FAMEs over the monounsaturated FAMEs which were present under the less harsh conditions. When solely methanol was used as a solvent along with sulfuric acid C16(0), C18(1) and C18(3) were the predominant FAMEs. When rapeseed oil was used instead of algal lipid a more varied FAME profile was observed, comparable to that under reflux acid transesterification, suggesting that the method is comparable to more conventional methods for transesterification. The use of higher amounts of algae would be required to corroborate this result for algae and take advantage of the improved experimental conditions. Interestingly, as mentioned above, the microwave conditions resulted in the production of FAMEs (48.4 % conversion without catalyst, *cf.* 65.9 % with H_2SO_4) even without the presence of catalyst, however in further preliminary work to ensure full transesterification of all lipids sulfuric acid was added.

Table 3:4 – Preliminary microwave extraction and transesterification conditions

Test Number	Hold temperature / ^{o}C	Hold time / minutes
AP1	80	2
AP2	80	10
AP3	80	20
AP4	80	120
AP5	80	10
AP6	80	10
AP7	80	10
AP8	80	10
AP9	120	2
AP10	120	10
AP11	120	20
AP12	120	120

To obtain maximum monounsaturated FAMEs further preliminary work was carried out at temperatures of 80 ^{o}C and 120 ^{o}C, rather than higher temperatures where saturated FAMEs were more predominantly observed. Literature[21] also observed that temperatures of between 80 and 95 ^{o}C yielded the highest lipid content, which suggests that the pressure produced aids the extraction or transesterification.

The *Anton Paar microwave synthesis monowave 300 reactor* and *autosampler MAS 24* was used with *G10 vials*; specialised 10 ml vials for use in the microwave under pressurised conditions. 3 ml $CHCl_3$ and 1.5 ml MeOH was used with 0.1 g dried *Chlorella emersonii*. To aid with transesterification and ensure the maximum FAMEs were obtained from the experiments sulfuric acid was added in excess (2 drops). Time periods at the hold temperature were decided to be 2, 10, 20 and 120 minutes. As can be seen in Table 3:4, AP5 – AP8 were used as a standard to observe the repeatability and reliability of this technique.

The results of the experiments in percentage of FAMEs produced and FAME profiles were obtained using GCMS/FID and are displayed in Figure 3:22. The four repeat experiments AP5, AP6, AP7 and AP8 can be seen in the centre of the graph (Figure 3:22) are quite varied in overall FAME content, 3.974 mg ± 21.48 %, whilst overall variability for all tests

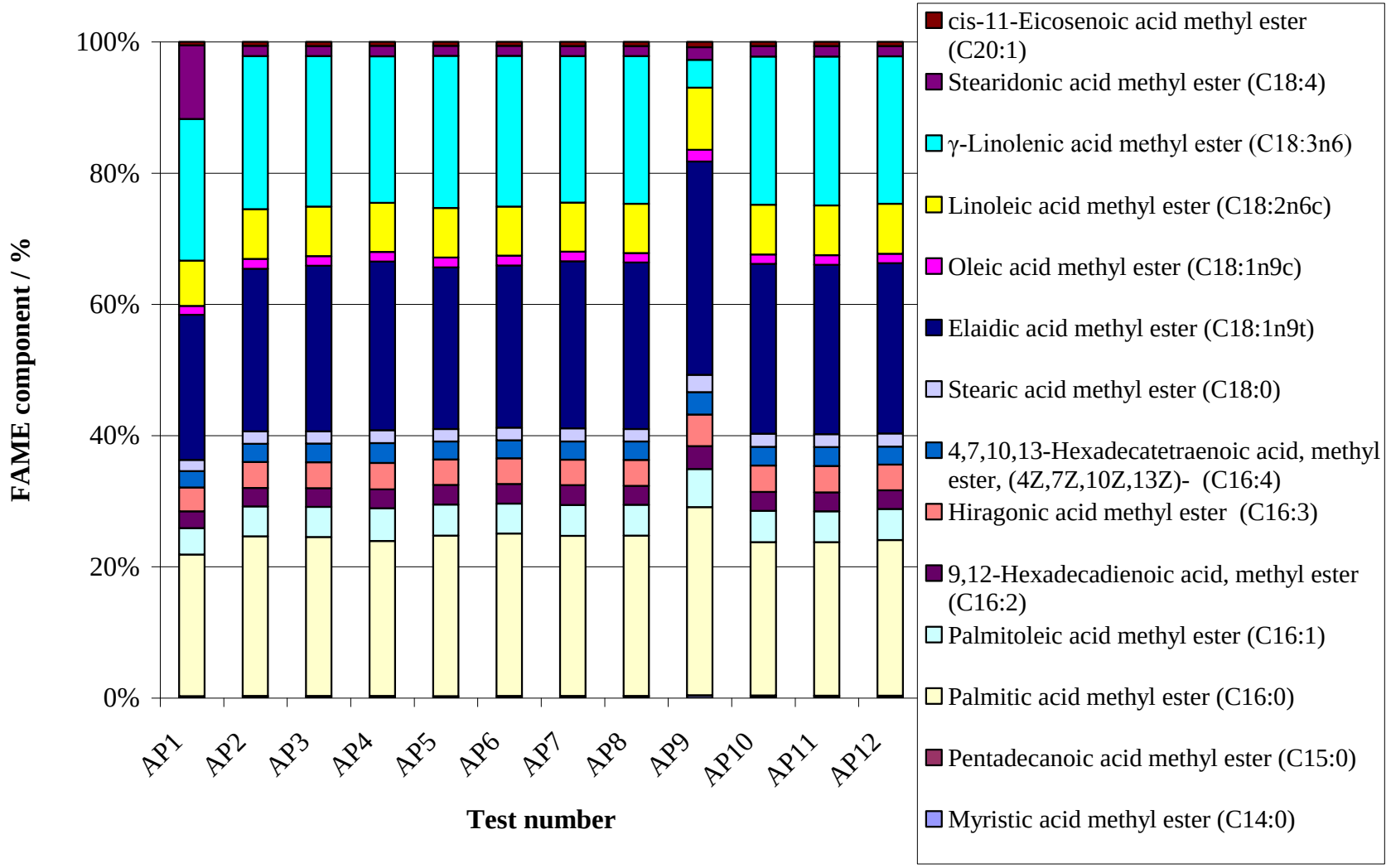

Figure 3:22 – Anton Paar microwave preliminary extraction and transesterification FAME distribution (Table 91)

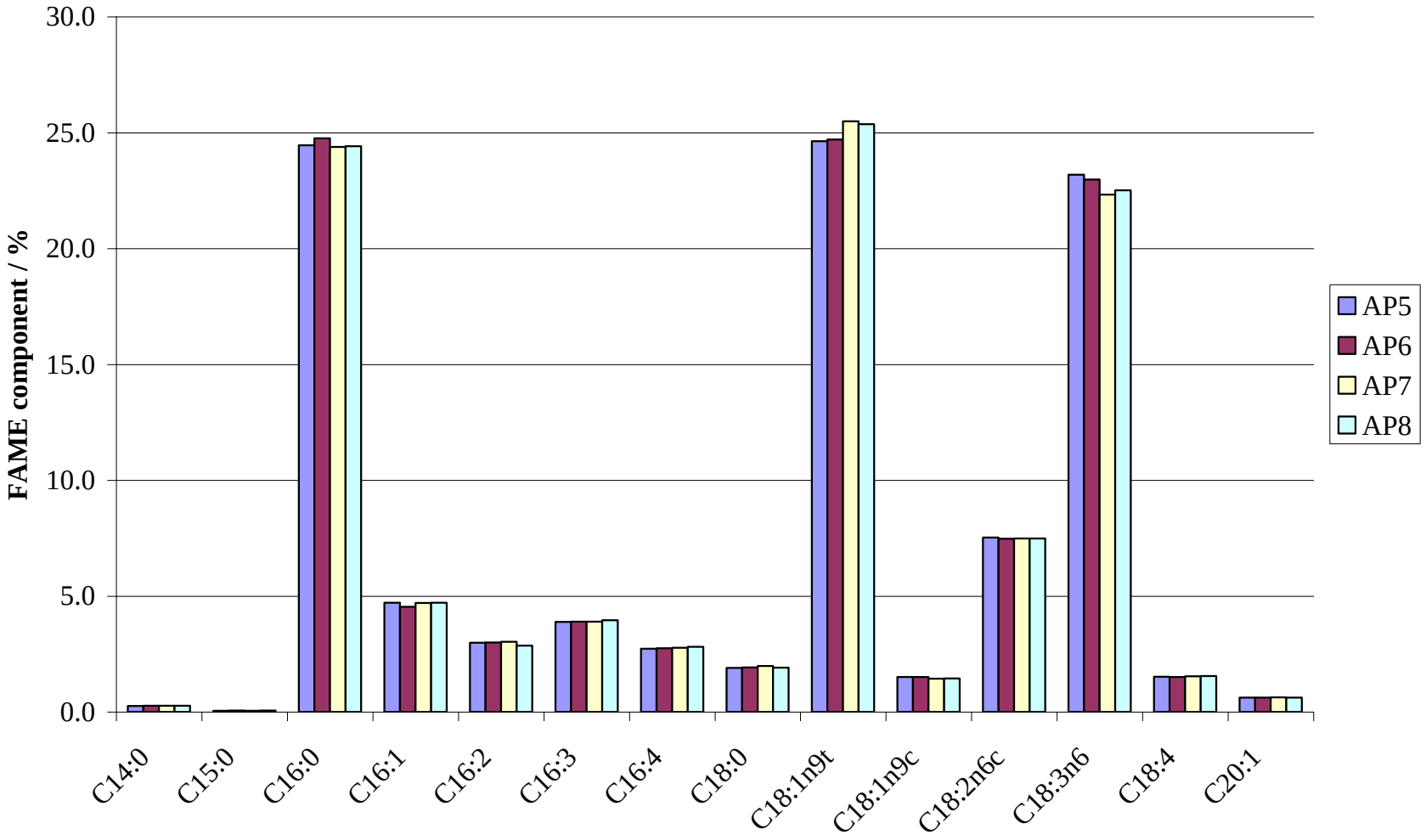

Figure 3:23 – FAME profile for control tests for the microwave preliminary experiments by percentage (Table 91)

was 4.403 mg ± 17.74 %. It is therefore FAME composition which is of more interest in this experiment due to the small samples tested which contributed to the 21.48 % variation in FAME yield. Regardless of the discrepancies in overall FAME amounts produced the FAME profiles for the control tests are extremely similar (Figure 3:23) which indicated the repeatability of the testing.

An increased length of hold time at the reaction temperature did not appear to significantly encourage lipid/FAME motility at either temperature of 80 ^{o}C or 120 ^{o}C (Figure 3:22). All of the tests had a standard deviation of 17.7 %, thus to reduce energy consumption and due to speed of reaction time, 2 minutes should be used as a hold time and the temperature should be changed to obtain the preferred FAME profile. At 80 ^{o}C when held for just 2 minutes (AP1) there is over 5 mg FAME extracted with 11.2 % of the FAME being C18(4) compared to 1.9 % when tested at 120 ^{o}C (AP9). At 120 ^{o}C for 2 minutes (AP9) the yields increased for C18(1) to 34.3 % compared to 23.5 % for 80 ^{o}C. For the higher temperature lipid extraction and transesterification there was 5 times less polyunsaturated C18(3) than at 80 ^{o}C, but a 7 % increase in saturated C16(0) compared to the lower temperature tests.

The extraction ability of the microwave requires further manipulation since an average of 4.40 % ± 0.78 % of the algal cells were extracted and transesterified into FAMEs using this technique. This is a rather low amount, however it is expected that it is due, in part, to the low amount of algae, 0.1 g, used and residual lipid on glassware remaining unanalysed. Microwave extraction is an improved extraction technique compared to sonication and Soxhlet extraction, as can be observed in Figure 3:24. In Figure 3:24 it is visible that there are varying amounts of each FAME extracted with each technique; the samples for each technique were taken from the same sample of algae. For example, with microwave extraction and concurrent transesterification there was increased C16(0) and C18(1) produced and C18(4) was observed as well as increased amounts of other unsaturated FAMEs such as C16(4). Whereas with Soxhlet extraction and subsequent acid transesterification there were an increased amount of shorter chain FAMEs formed whilst maintaining the general predominance of C16(0), C18(0), C18(1), C18(2) and C18(3). This suggests that shorter chain triglycerides were more easily extracted and therefore transesterified using Soxhlet extraction and subsequent acid transesterification. Overall the use of microwaves under a sealed atmosphere allowed for more unsaturated and generally more FAMEs to be extracted. Whether the FAMEs are produced *in situ* within the algae

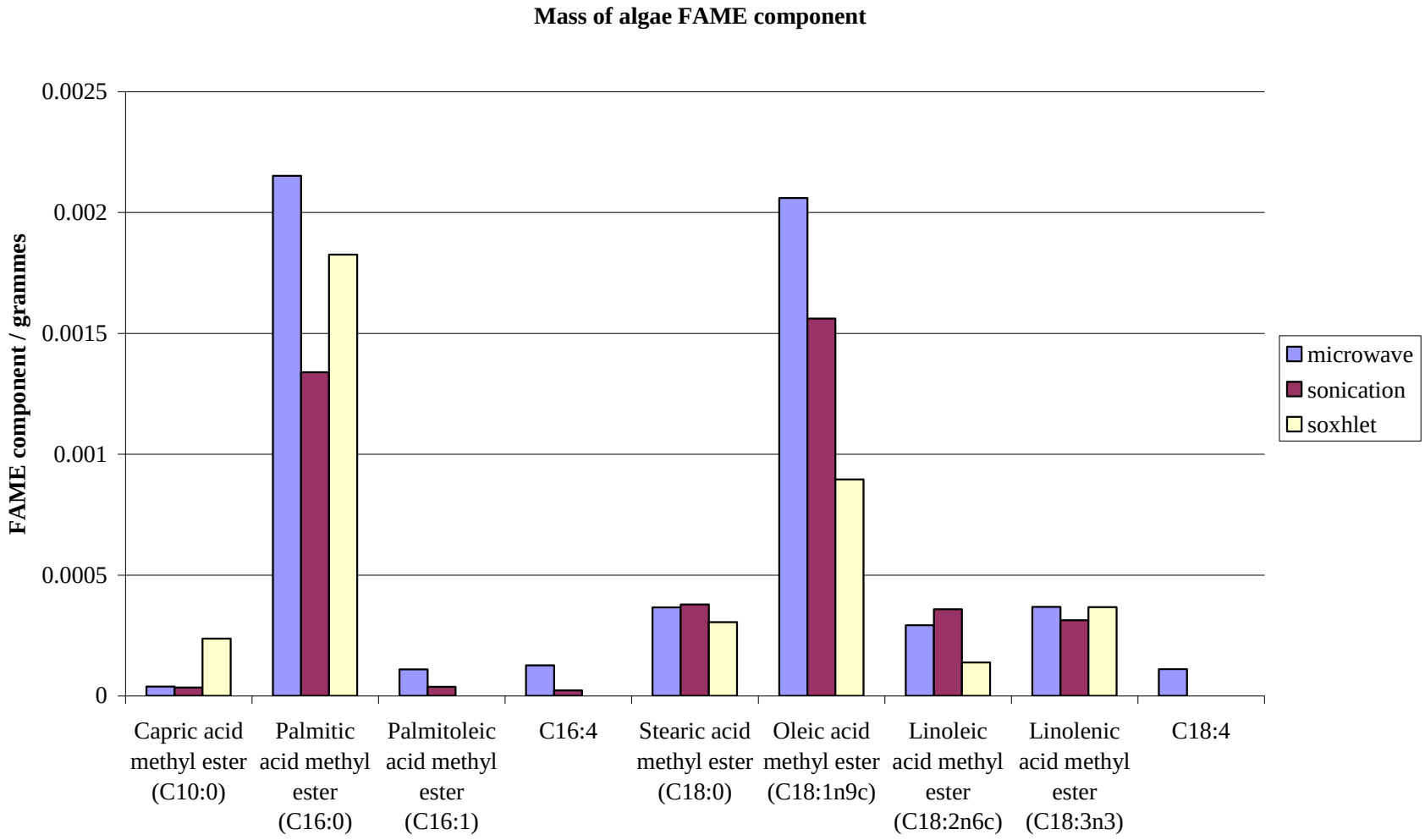

Figure 3:24 – Comparison of extraction techniques for 0.1 g *Chlorella emersonii* (Table 92)

cell and then extracted or if the triglycerides permeate the algae cell walls and are subsequently transesterified is unknown. Due to the higher amounts of FAME produced and the more bulky construction of these FAMEs it is proposed that the triglycerides are first transesterified inside the cells and then permeate the cell wall, since they are less bulky than the more complex triglyceride structures.

3.10 Summary

Algal-derived biodiesel has positive qualities for the fuel industry and contains lipid soluble anti-oxidants which hinder the oxidation of the highly poly-unsaturated chains formed. The determination of FAME profile for the resultant FAMEs was carried out, initially by mass spectrometry and later able to be quantified by GCMS/FID. Unusual non-terrestrial FAMEs such as C16(4), C17(4) and C18(4) have been observed within the FAME profiles; this has been observed in literature[1, 22], but the levels and change in percentage of these unusual highly unsaturated triglycerides have not been studied.

Initially the lipid percentage in *Chlorella emersonii* was established to be higher than that for other fast-growing *Chlorella* spp. including *Chlorella vulgaris* using NMR spectroscopy; corroborating reports in literature.[23] Extraction of lipid from *Chlorella emersonii* was determined to be most successful using chloroform: methanol in a 2:1 ratio, which was also found for some species in literature too[24]; the use of acid catalysed transesterification was found to be most reliable for the conversion of lipid to FAMEs.[25]

Length of cultivation and nutrient availability had an effect on the subsequent FAME profile produced from the algal lipid. The stage of growth of the algae also had an impact on the profile of the triglycerides. To obtain maximum algal lipids from a culture it is ideal to harvest around 120 hours before total agglomeration of the cell culture (Figure 3:17) regardless of culture size. The reduction of nitrate levels for *Chlorella emersonii* has been shown to increase lipid percentage in the algal cells, however this does not translate to increased lipid amounts in the samples overall, due to reduced cell proliferation (sections 3.5 – 0). Literature published by the *University of West England*[4, 16b] showed that there was an increase in lipid percentage for *Chlorella emersonii* and *Chlorella vulgaris* however there is no mention of overall growth or lipid amount. The decrease in growth rate and therefore in lipid yields is indicated by Kumar *et al.*[13]and Lardon *et al.*[26]; this has been attributed to the storage of energy in lipid form when the algae is under stress. The review

by Kumar *et al.*[13] and the policy analysis by Lardon *et al.*[26] contain no data for the assumption that growth rate will be affected or that lipid yield will increase under the stress conditions of reduced nitrate concentration, they are mainly focussed on the life cycle analysis of the entire algal fuel process; a specific species of algae is not indicated. It therefore seemed important to be able to quantify the assumption that lower nitrate concentration reduces overall algal growth and lipid output, despite the increase in lipid amount for a specific species of algae, in this case *Chlorella emersonii.* This thesis also highlights that increased cultivation time alongside Bold's Basal level nitrate supply leads to high levels of overall lipids, whilst a reduction in phosphate levels with increased cultivation time leads to maximum lipid output, according to Figure 3:15f which is due to a rise in unsaturated lipids. The fluctuation in unsaturated lipids has been corroborated in literature by Converti *et al.*[27] as for *Chlorella vulgaris* no significant changes were observed in resultant C16(0) yields, however an increase in C18(3) was observed under lowered nitrate levels.

For phosphate and iron concentrations the ideal for maximal lipid output of the algae with consistent, reliable and usable fuel is the standard Bold's Basal growth medium amounts, which are optimised for green algae. Wang and Lan[15a] optimised the lipid production of *Neochloris oleoabundans*, grown in a modified Bristol medium, using the Box-Behnken design of experiments. The conclusions were that in initial testing the micronutrients were in sufficient excess as not to have an effect on cell growth or lipid accumulation; however the phosphate concentration has a significant effect on cell growth, nitrate concentration has a significant effect on lipid accumulation and the magnesium sulphate/ferrous chloride concentration has a significant effect on both cell growth and lipid accumulation. There is also a synergetic effect of phosphate and nitrate concentration upon cell growth. For the nitrate concentration, lipid accumulation by mass was highest between the normal levels for the modified Bristol medium and lowered levels, and significantly reduced at increased levels. For magnesium sulphate/ferrous chloride levels the lipid accumulation by mass is increased at between normal levels for modified Bristol medium and increased levels, becoming significantly reduced at lowered levels.[15a] These results from literature can be used for comparison with the changes in lipid accumulation and algal growth observed for *Chlorella emersonii* cultured in Bold's Basal medium, however the species specificity of such changes as well as the difference in media highlight that caution is required with generalisations. The Wang and Lan journal article[15a] did not analyse the resultant FAME

profile of the algal lipid under the different growth conditions or take into account the length of cultivation; the FAME profile of the transesterified lipid and the amount of lipid yielded was dependent on the length of cultivation and the nutrient availability.

Carbon dioxide supplementation to air was critical for enhanced *Chlorella emersonii* growth, as observed by Carvalho *et al.*[28]. The addition of a supplementary carbon source to the phototrophic algae, *Chlorella emersonii,* was not required for increased lipid production; carbon dioxide at 3 – 5 % was sufficient and required for healthy cell proliferation.

Microwave technology has the potential to be more economical and quicker than Soxhlet extraction and subsequent transesterification. The microwave technology extracted more lipid than sonication or Soxhlet techniques, and the lipid extracted was transesterified into fatty acid methyl esters. It was also elucidated that shorter chain FAMEs are more easily extracted, either in the form of triglycerides or pre-transesterified into FAMEs, whichever extraction technique was used, this has not been previously reported. The use of microwave technology to extract and transesterify lipid into FAMEs from algae has been reported by Wahlen *et al.*[29] as well as the investigation of the extraction of wet algae – however this is still largely unstudied. In the experiments carried out higher yields were obtained due to the addition of chloroform into the reaction mixture which had not been previously trialled.

3.11 References

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4 Experimental

4.1 General procedures

All starting materials and solvents were purchased from Sigma Aldrich, Fisher or Acros Organics and used as received. Oils used were purchased from local supermarkets where possible, except for palm oil which was obtained from Ghana, coconut oil which was supplied by Sigma Aldrich and waste oil which obtained from the University canteen. No pre-treatments were applied to the oils. Algae, *Chlorella vulgaris, Chlorella VT-1* and *Chlorella emersonii* (2 x growth conditions), for preliminary experiments and screening were obtained from the *University of West England* (UWE). Subsequent algae, for all experiment excluding the initial algal species and solvent screening, were sourced from the *Culture collection of algae and protozoa* in Oban, Scotland, then maintained and stored in the Department of Biology, *University of Bath*. These cultures were used for subsequent experiments. The drying of oils and biodiesel for quantification, and other appropriate manipulations were carried out under inert conditions using a Schlenk line. Where appropriate solutions and cultures were sterilised using standard autoclave conditions.

4.2 Lipid extraction

Soxhlet extraction technique was used with 50 mL methanol and 100 mL chloroform under reflux until the extraction solvent remained colourless. After complete extraction the lipids were dried using a vacuum line.

4.3 Transesterification of lipids

20 mL methanol and 1 mL H_2SO_4 were used for transesterification of the lipids into fatty acid methyl esters (FAMEs) when unquantifiable lipid was extraction from the algae. If the lipid amount was quantifiable 6 mole equivalents of methanol and 2.5 wt% H_2SO_4 was used. Reflux conditions were maintained for 4 hours. The reaction was quenched with deionised water and separation was achieved using an organic wash with chloroform. After complete separation the FAMEs were dried using a vacuum line and analysed using ^1H NMR spectroscopy, GCMS or viscometer.

4.4 Nuclear magnetic resonance analysis

^1H NMR spectroscopy was used to analyse the yield of FAME produced by dissolving the sample in $CDCl_3$ (chemical shifts of residual protio solvent resonances referenced to

$CHCl_3$ δ 7.26). *Bruker 250* and *300 MHz NMR spectrometers* were used for the analyses at 293 K.

Method 1: The integral value of the methoxy group of the FAME was compared to that of the remaining proton signals adjacent to the glycerol backbone of the triglyceride; this technique was first reported by G. Knothe.[1] Calculating FAME production from NMR spectra deviates from HPLC values by 3 %.[1] Figure 4:1 shows the change in spectra for algal lipid (red) which has been transesterified into FAMEs (blue). The disappearance of the glyceride backbone multiplet at 4.0 – 4.2 ppm (H) led to the appearance of the singlet peak at 3.6 ppm (G) for the methoxy moiety on the three resultant FAMEs (produced from each triglceride).

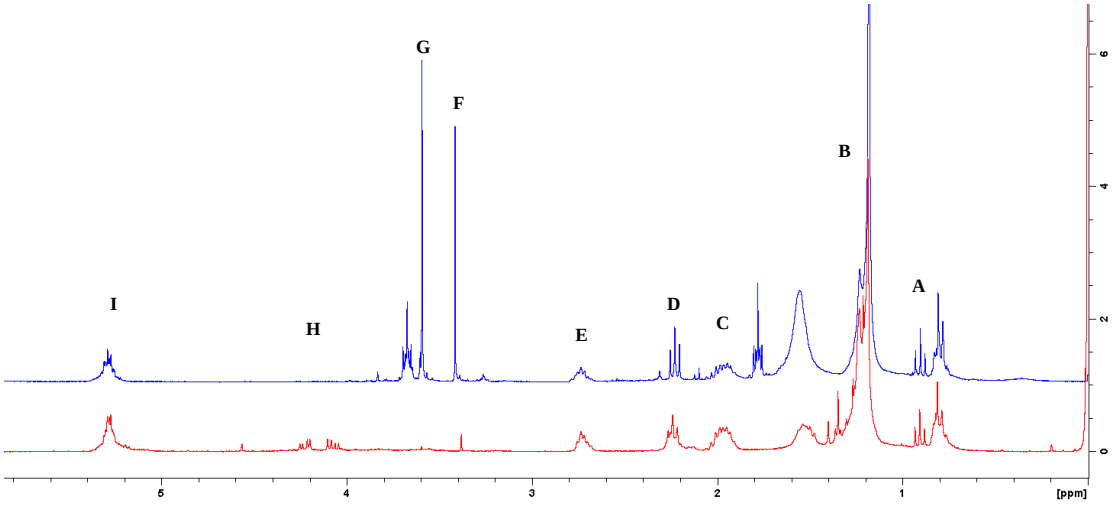


Figure 4:1 – ^{1}H NMR in $CDCl_3$ of *Chlorella emersonii* biodiesel (blue) and *Chlorella* emersonii lipid (red): A – C$\underline{H}_3$, B – C$\underline{H}_2$ alkyl chain, C – C$\underline{H}_2$CH=CH, D – O_2CC$\underline{H}_2(CH_2)_x$, E – CH=CHC$\underline{H}_2$CH=CH, F – OC$\underline{H}_3$ methanol, G – OC$\underline{H}_3$ methoxy moiety on FAME, H – C$\underline{H}_2$C$\underline{H}$C$\underline{H}_2$ glyceride backbone on lipid, I – C$\underline{H}$=C$\underline{H}$CH$_2$ (method 1)

Method 2: Alternatively the actual mass of FAME produced was determined by the addition of a known amount of benzaldehyde to the NMR sample. Figure 4:2 shows the NMR of biodiesel produced from *Chlorella emersonii* which was grown at pH 6.5. The benzaldehyde peak was observed at 10 ppm and from this and the peak at 3.6 ppm it can be determined that 65.3 mg of biodiesel were produced from the sample. The triglyceride multiplet which comes around 4.2 ppm was used to compare with the peak at 3.6 ppm to determine that 93.4 % conversion of triglycerides to biodiesel has occurred under acid catalysed conditions.

Terminal aliphatic groups are observed around 0.8 – 0.9 ppm (A), methylene protons at

between 1.1 – 1.3 ppm (B). At ~1.5 ppm the adjacent methylene protons to the ester are observed, allylic protons (those next to the double bonds) are seen at 1.9 – 2.1 ppm (C). Between 2.2 and 2.3 ppm (D) the protons next to the ester are observed and at 2.6 – 2.8 ppm (E) protons between pairs of double bonds are seen. At 3.6 ppm (G) the terminal methyl ester group is observed alongside the residual methanol peak (F) whilst between 5.2 and 5.4 ppm (I) the olefinic protons are observed and at 7.26 ppm the reference peak for undeuterated chloroform is observed. The multiplet for the 5 protons in the glyceride backbone of any residual triglyceride is seen at 4.0 – 4.2 ppm (H).

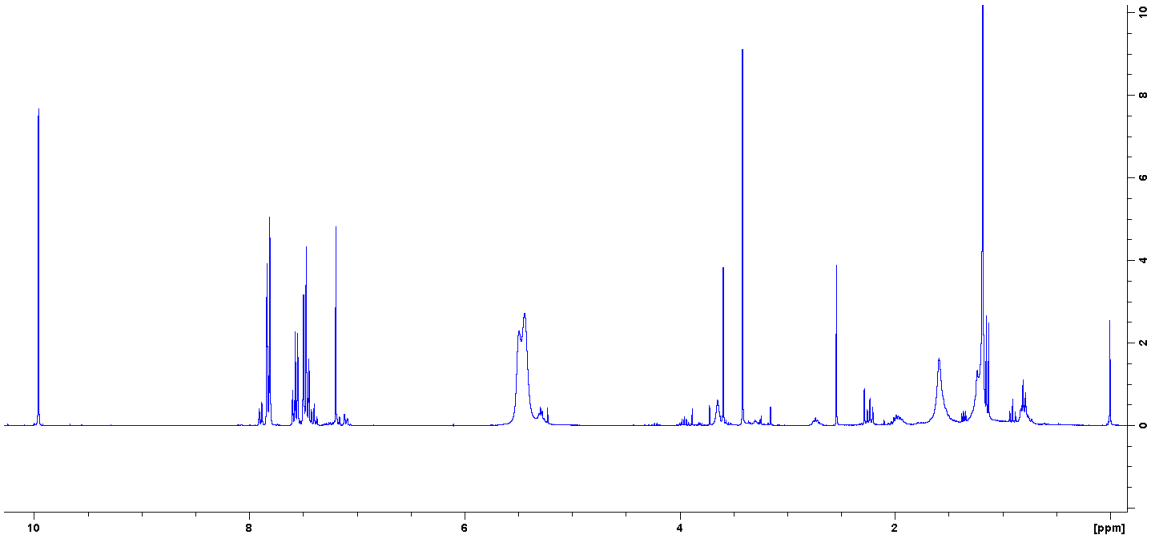


Figure 4:2 – NMR of biodiesel produced from *Chlorella emersonii* grown at pH 6.5 (method 2)

4.5 Mass spectrometry

FAME samples were dissolved in methanol with 0.05 % sodium methoxide to aid solubility. Using a *micrOTOF ESI-TOF (electrospray time of flight)* mass spectrometer coupled to an *Agilent 1200* liquid chromatogram 10 µl of sample was injected into a 0.3 mL min^{-1} solvent phase of 30: 70 water: acetonitrile. A positive loop injection scanning between 50 and 1500 m/z was used. Analysis and peak identification was carried out using *Bruker Daltonics Compass DataAnalysis 4.0*.

4.6 Gas chromatography mass spectrometry (GCMS)

GCMS analysis was carried out on an *Agilent 7890A Gas Chromatograph*. The GC was equipped with a capillary column (60 m × 0.250 mm internal diameter) coated with *DB-23* ([50%-cyanpropyl]-methylpolysiloxane) stationary phase (0.25 µm film thickness). A He mobile phase (flow rate: 1.2 mL min^{-1}) was used and the GC was coupled with an *Agilent 5975C inert MSD with Triple Axis Detector*. Biodiesel samples were dissolved in known

amounts of dioxane (300 µL – 2 mL). 1µL of the biodiesel sample solution was loaded onto the column which had been pre-heated to 150 °C. This temperature was held for 5 minutes whereupon the column was heated to 250 °C at a rate of 4 °C/min, then held for 2 minutes. The column which has a very strong dipole characteristic was purchased to give good resolution of C16 and C18 FAME isomers; the length was chosen to increase resolution.

For calibration of the column FAME mixture was purchased from *Sigma Aldrich* (*Supelco*), C4-C24, 67.10 mg. This FAME mixture was dissolved in 1,4-dioxane (10 mL) to 6.71 mg mL^{-1} and diluted to concentration shown in Table 4:1. The resultant calibration data is shown in Figure 4:3, as can be observed there was little error over the 5 readings, except for 2 readings at the highest concentration. The reason for this increased error was unknown and therefore explained as experimental error due to different prominence of elution. The readings which were particularly high were due to higher percentage (6 %) of C16(0) in the standards mixture, and the 4 % of C18(0) eluting more prominently than the other FAMEs. The error bars have only been shown for the highest concentration of calibration tests run, for clarity.

Table 4:1 – Calibration concentrations and weights for FAME GCMS

Concentration / mg mL^{-1}	**Mass injected / mg**	**2 % / ng mL^{-1}**	**4 % / ng mL^{-1}**	**6 % / ng mL^{-1}**
0.0671	2.03×10^{-3}	0.0407	0.0813	0.1220
0.671	0.020	0.407	0.813	1.22
3.355	0.102	2.033	4.067	6.10
6.71	0.203	4.07	8.13	12.2

Each calibrant was run five times and each component was identified on the mass spectrometer using the *NIST database*. An example of the separation of the calibrant FAME constituent separation is shown on the spectrum in Figure 4:4, with the highlighted clear separation of the C18 region in Figure 4:5, allowing cis and trans isomers to be distingushed. The comparable integral of the peak was measured using flame ionisation detector, since FID is more quantitative than MS, which is more diagnostic. The integrated area under each of the peaks was plotted onto a calibration graph to allow for quantification of subsequent runs (Figure 4:3).1 µl of each sample was injected with a split

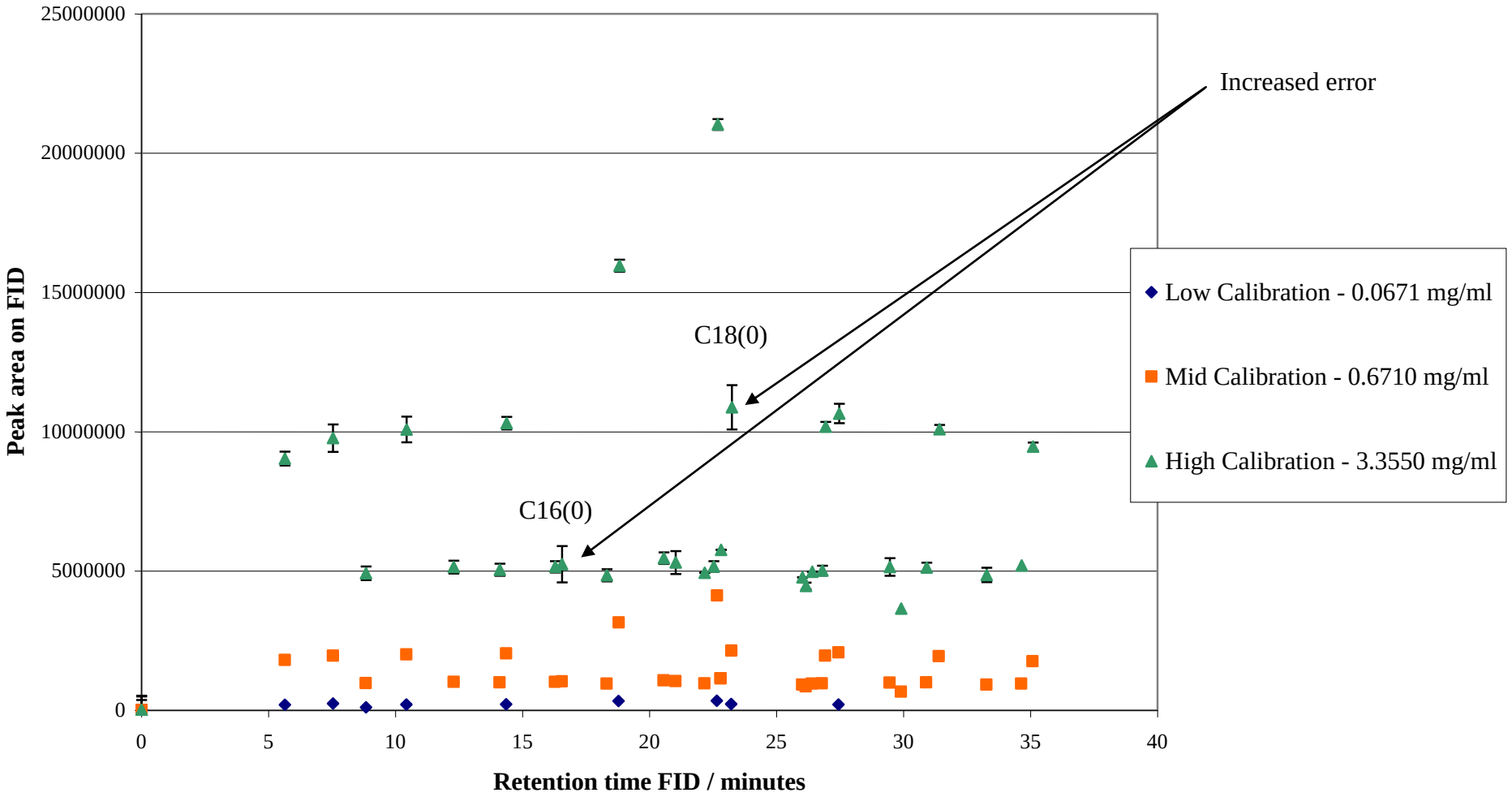


Figure 4:3 – Calibration data for Supleco FAME trace

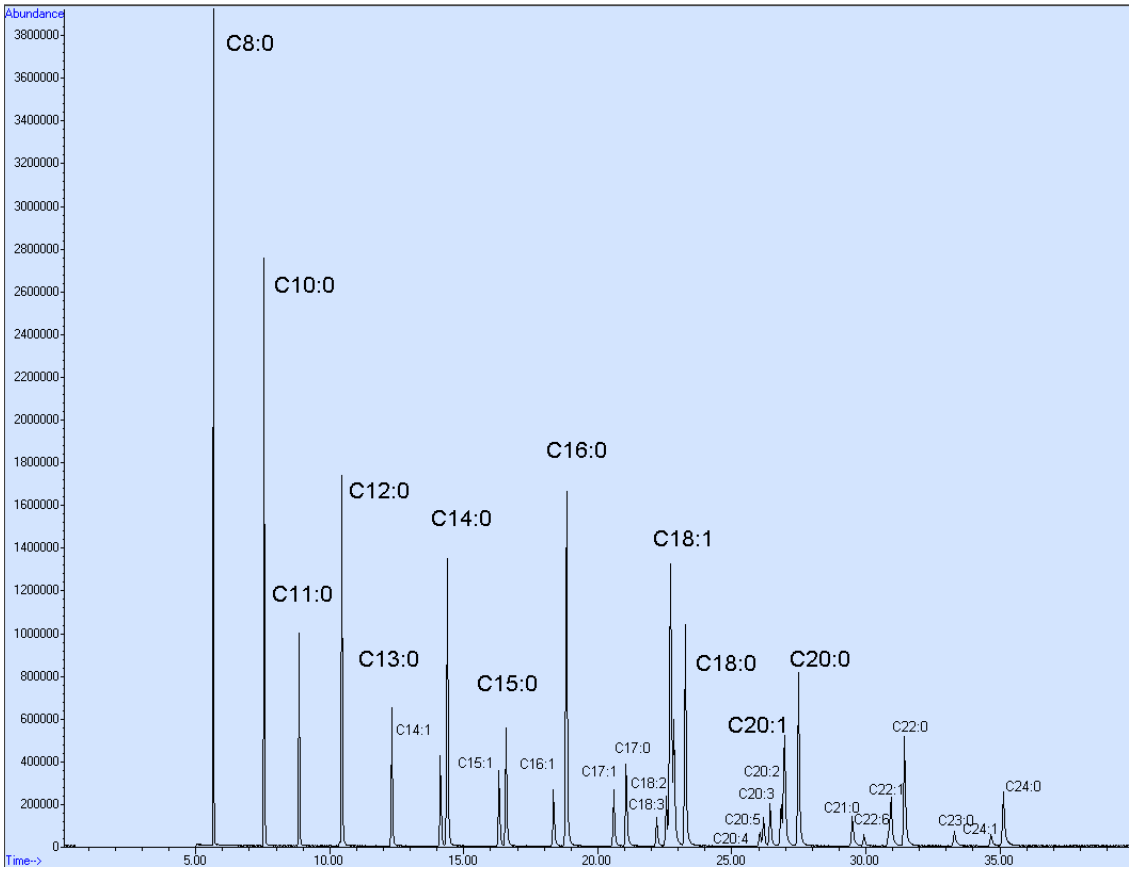


Figure 4:4 – Calibration GC-FID spectrum of FAME constituent separation on a DB-23 column

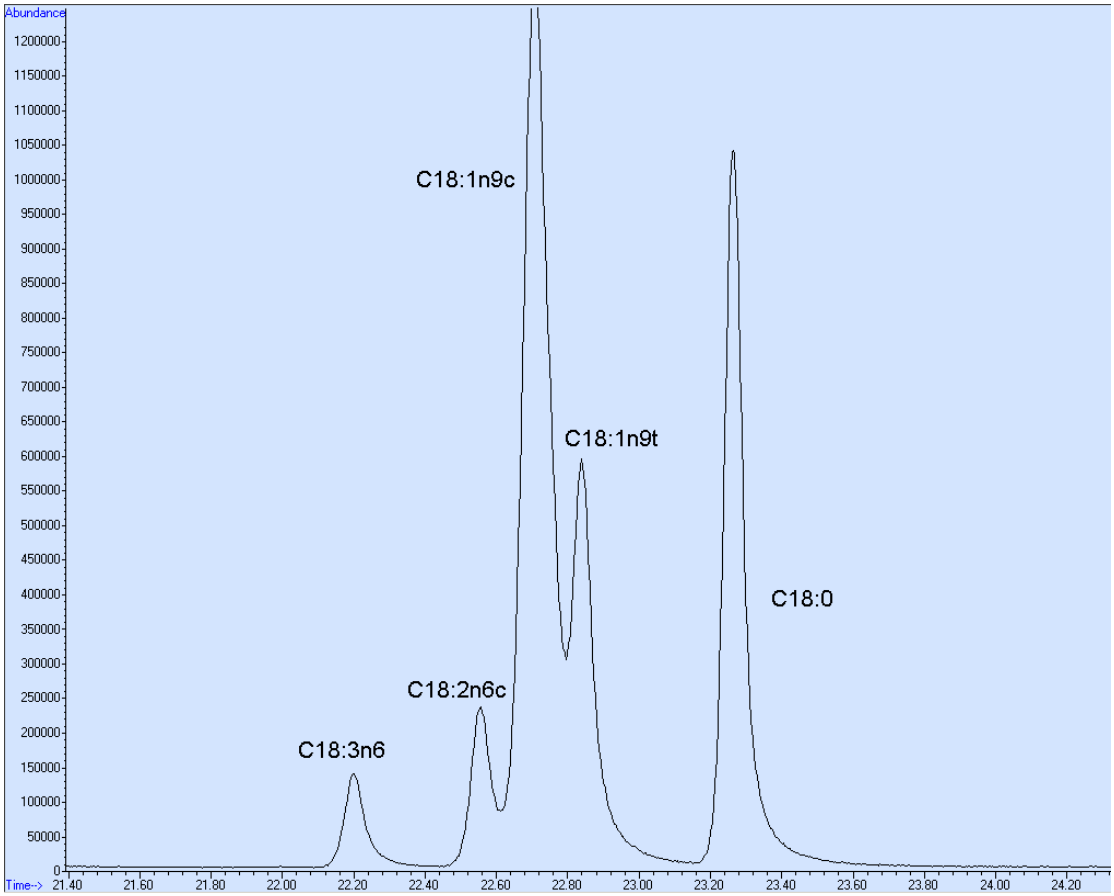


Figure 4:5 – C18 region highlighting clear separation in GC-FID spectrum for calibrant FAMEs

ratio of 10: 1 (*i.e.* 10 parts waste to 1 part through the column) and a 3 way split to the MS, FID and TCD detectors. The masses injected are shown in the second column of Table 4:1. All peaks which were detected as 0.001 % or more of the total correlated area in the FID detector were analysed as possible FAME and other components. The injected mass of each individual component, dependant on the percentage present in the FAME sample is shown in Table 4:1. Lower molecular weight FAMEs could not be identified using the MS as they eluted before the MS was switched on. The delay in the MS detector analysis was due to the solvent elution causing potential damage to the detector. *Enhanced Data Analysis*, was used for examining the mass spectra and outputted flame ionisation detection traces. The identities of specific peaks were made using comparative techniques between calibration peaks, in comparison with the *National Institute of Standards and Technology (NIST) 2005 mass spectral library (NIST, Gaithersburg, MD)* and the mass spectra of individual peaks. The mass range scanned was 20 – 2000 m/z using a nominal m/z value of 272 at a rate of 8 scan s^{-1}. The variance in peak area and elution time on the FID was due to differing amounts of each FAME component being present in the calibrant. In addition to the amount of FAME component the FID peak area and elution time were also dependant upon the ionisation, the size and the shape of the component. The chain length of the FAME component and the degree of saturation, as well as branching, all affect the speed of the components travel through the column and its eventual detection by the FID. More information about the GCMS/FID analysis and calculations can be found in Appendix 4.

4.7 Microwave technique

An automated microwave reactor was trialled and purchased for further development of algae extraction and transesterification technique. This technique has good potential for obtaining useful, high-value products from other biological matter such as lignin.

Using an *Anton Paar microwave synthesis monowave 300 reactor* and *autosampler MAS 24*, temperature was able to be controlled, as was the length of time at a particular temperature. Pressure could be checked using the logged data, which included temperature, pressure and power used over the course of each reaction (section 3.9). Samples were made up in the vials supplied, which were of size 10 mL or 25 mL (G10 or G25 respectively), the caps were used to allow for high pressures to be obtained – up to 300 bar. After processing the algae reaction mixture required filtering to remove remaining algal solids

(since liquid lipids and FAMEs were of particular interest) and subsequent removal of solvent under vacuum.

4.8 Acid value

A titration technique from *Lubrizol* was used to quantify the acid value and thus the free fatty acid content of waste oil obtained from Level One canteen, *University of Bath*. 0.1 M of standardised potassium hydroxide was titrated against 1.0 g of waste oil dissolved in 50 mL neutralised isopropanol with 5 drops of phenolphthalein indicator solution added. A base line titration was carried out, and followed by three titrations. The acid value was calculated for all three titrations and the average taken.[2] The formula for acid value is as follows:

$$\text{Acid value} = \frac{\text{mL KOH x N x 56.1}}{\text{weight of sample (g)}}$$

4.9 Bold's Basal growth medium

Bold's Basal growth medium was made up from 6 major stock solutions and 4 minor stock solutions, see Table 4:2. These stock solutions were made up as shown in Table 4:2 using Millipore water; they were then autoclaved to ensure sterility and preservation whilst on the shelf. 10 mL each of stocks 1 – 6 and 1 mL each of stocks 7 – 10 were added together, this solution was then topped up to 1 litre with Millipore water and neutralised to around pH 6.5 using 0.1 M sodium hydroxide solution. The growth medium was then autoclaved for sterility. Autoclave conditions were high pressure saturated steam at 121 °C for 15 minutes. This was repeated for the amount of growth medium required for each experiment.

4.10 Cell counting

For cell counting 10 μL of growth medium from the growth vessel was pipetted onto a haemocytometer slide under sterile conditions. The number of algae cells present was then counted by eye using a *Nikon Eclipse TE 2000-5* microscope. In later experiments an automated haemocytometer was used for cell counting, the *Millipore Guava Easycyte Flow Cytometer*. Centrifugation was carried out using a *Beckman Coulter Avanti J-25 centrifuge* for 4 minutes at 4 ^{o}C run at 4500 rpm for samples larger than 1 litre and using a *Beckmann Coulter Spinchron^{TM} DLX Centrifuge* for 4 minutes at 5000 rpm for samples smaller than 1 litre.

Table 4:2 – Bold Basal stock solution concentrations

Stocks		**Per 400mL / g**
1	$NaNO_3$	10.0
2	$MgSO_4.7H_2O$	3.0
3	NaCl	1.0
4	K_2HPO_4	3.0
5	KH_2PO_4	7.0
6	$CaCl_2.2H_2O$	1.0
7 Trace elements solution (autoclave to dissolve)		**Per L / g**
	$ZnSO_4.7H_2O$	8.82
	$MnCl_2.4H_2O$	1.44
	MoO_3	0.71
	$CuSO_4.5H_2O$	1.57
	$Co(NO_3)_2.6H_2O$	0.49
8	H_3BO_3	11.42
9	EDTA	50.0
	KOH	31.0
10	$FeSO_4.7H_2O$	4.98
	H_2SO_4 (conc)	1.0 ml
Medium		**Per L / mL**
Stock solutions 1 – 6		10.0 (each)
Stock solutions 7 – 10		1.0 (each)

4.11 Inoculation

Chlorella emersonii were stored on agar plates and kept at room temperature in a state of stasis until required. To prepare an inoculum 250 mL of Bold's Basal medium was made up from standard solutions as described in section 4.9. This solution was transferred into the inoculum growth vessel covered which was with aluminium foil and autoclave tape. The growth vessel containing the growth solution was autoclaved at 121 ^oC for a 15 minute cycle. Once the solution had cooled, a smear of *Chlorella emersonii* was introduced from one of the agar plates, and the vessel re-covered with the aluminium foil. This procedure was carried out underneath a sterilised flow hood to prevent contamination with the necessary aseptic techniques employed. The inoculums were grown initially in a Sanyo incubation chamber with 12/12 hour light/dark regimen at 25 ^oC on a shaker plate. Later, with the development of a designated algae growth room and the introduction of sparging, 3 % carbon dioxide enriched air into the growth vessels, a light regimen of 18:6 hour day: night at a light intensity of 300 μmoles s^{-1} light m^{-2} at 25 ^oC was implemented. This lighting

regimen together with the sparging of carbon dioxide was found to give optimal *Chlorella emersonii* growth.

For the inoculation of the Box-Behnken (design of experiments) growth condition experiment, pre-autoclaved growth vessels with specifically tailored Bold's Basal medium, and other additives if required, were prepared and autoclaved in storage bottles. These specific nutrient Bold's Basal mixes were opened in a sterilised flow hood alongside the inoculum culture and a specified amount of inoculant was added, depending on the individual growth test. For the photobioreactor experiments, 5 L of autoclaved Bold's Basal solution was added to the photobioreactor. To this solution the inoculation culture was added and the remaining volume topped up to level with 2 L of Bold's Basal solution.

4.12 Design of experiments – effect of nutrients and time on algal growth

Chlorella emersonii grown for 4 days with 3 % carbon dioxide in air sparged through 2.5 Bold's Basal growth medium in a growth room with 18: 6 hours (light: dark) of 300 µmoles/s/m^2 white light at 25 ^oC. Sterility was ensured by autoclaving all equipment and growth media before use. After 4 days growth in the parent culture the algae were divided (1 mL of parent culture) into 24 specified environments. Each of the environments had different nutrient levels and times of growth as shown Table 4:3. Four standard growth environments provided the control batch. The second stage of the experiment was carried out in vials containing 15 mL of media, sufficiently small to allow for the same light intensity and movement to be experienced by each sample.

Other nutrients which are made up to 1 litre with the nutrients in Table 4:3 are at the amounts of 10 mL L^{-1} - $MgSO_4.7H_2O$, NaCl, $CaCl_2.2H_2O$, and at amounts of 1 mL L^{-1} - $ZnSO_4.7H_2O$, $MnCl_2.4H_2O$, MoO_3, $CuSO_4.5H_2O$, $Co(NO_3)_2.6H_2O$, H_3BO_3, EDTA and KOH – all according to the concentrations of the Bold's Basal growth medium as reported in Table 4:2.

Agitation was supplied in the inoculation culture by twice daily rigorous shaking alongside the sparged gas, whereas for the individual design of experiment growth vials shaking was provided by an adapted shaker within the *Sanyo incubation chamber* where growth occurred. The four control samples were placed on the corners which allowed them

(25 – 28) to experience the same light conditions as each other and for all other samples (1 – 24) to experience identical light conditions as each other to allow for sufficient control of experiential light.

Table 4:3 – Design of experiments (nutrients) variables

	Nitrogen	**Phosphate**		**Iron**	**Time**
Test	**$NaNO_3$**	**K_2HPO_4**	**KH_2PO_4**	**$FeSO_4.7H_2O$**	
	mL /L	**mL / L**	**mL / L**	**mL / L**	**days**
1	5	10	10	0.5	5
2	5	10	10	1.5	5
3	15	10	10	0.5	5
4	15	10	10	1.5	5
5	10	5	5	1	3
6	10	5	5	1	7
7	10	15	15	1	3
8	10	15	15	1	7
9	5	10	10	1	3
10	5	10	10	1	7
11	15	10	10	1	3
12	15	10	10	1	7
13	10	5	5	0.5	5
14	10	15	15	0.5	5
15	10	5	5	1.5	5
16	10	15	15	1.5	5
17	5	5	5	1	5
18	5	15	15	1	5
19	15	5	5	1	5
20	15	15	15	1	5
21	10	10	10	0.5	3
22	10	10	10	0.5	7
23	10	10	10	1.5	3
24	10	10	10	1.5	7
25-28	10	10	10	1	5

The work up of all samples was the settling of algae after the set time point and removal of media, Soxhlet extraction of lipids (see section 4.1), removal of solvent under vacuum, transesterification of the lipids into FAMEs (see section 4.3). The FAMEs were then separated via organic solvent extraction from the reaction mixture, the solvent removed under vacuum and dissolved in a known amount of 1,4-dioxane for analysis on the GCMS/TCD/FID (see section 4.5).

4.13 Experimental design for mixotrophic study

2.5 L of autoclaved Bold's Basal medium was inoculated with *Chlorella emersonii* and grown for 4 days with sparged 3 % carbon dioxide (in air) in growth room (at 25 ^{o}C with 300 μmoles/s light / m^2 for 18 hours per day).

After 4 days of inoculant growth, ten 500 mL conical flasks were filled with 150 mL Bold's Basal medium whereupon 2 mL inoculation culture was added along with the additional carbon sources for each of the samples, as recorded in Table 4:4.

Table 4:4 – Additional carbon sources for mixotrophic experiment

Test number	**Compound added**	**Mass of added carbon source (2 mol % / L) / g / 250 mL**
1-5	Glucose (pure)	0.90
6	Glycine (pure)	0.38
7	Glycerol (pure)	0.46
8	Spent algae biomass	2.01
9	Ethyl acetate (pure)	0.44
10	None	None

Growth of *Chlorella emersonii* was carried out in the 10 test vessels with added sparged 3 % carbon dioxide (in air) using a splitter in growth room (at 25 ^{o}C with 300 μmoles/s light / m^2 for 18 hours per day) for 5 days.

The samples were centrifuged for harvesting, a Soxhlet extraction was performed and the resulting lipid dried under vacuum, whereupon acid transesterification was undertaken and the organic layer separated and dried for analysis by GCMS/TCD/FID.

4.14 Experimental design for toxicity studies

1 L of autoclaved Bold's Basal medium was inoculated with C*hlorella emersonii* and grown for 4 days with sparged 3 % carbon dioxide (in air) in growth room (at 25 ^{o}C with 300 μmoles/s light / m^2 for 18 hours per day).

After 4 days of inoculation growth ten 500 mL conical flasks were filled with 150 mL Bold's Basal medium, 2 mL inoculation culture was added along with the additional construction material from the photobioreactor for each of the samples, as recorded in Table 4:5. They were sparged with 3 % carbon dioxide in air using the splitter as for

section 4.13, and grown under 300 μmoles/s light / m^2 for 18 hours per day conditions at 25 ^oC.

Table 4:5 – Construction materials added for toxicty experiment *No test: no algae grown, contained Millipore water to ensure equal pressure of the gas splitter and lower contamination risk

Test number	**Compound added (thickness)**	**Amount/contact area of construction material added / 250 mL**
1-3	None	None
4	Neoprene rubber (3 mm)	1 cm^2
5	Neoprene rubber (3 mm)	2 cm^2
6	Neoprene rubber (3 mm)	4 cm^2
7	Silicon grease	1 g
8	PTFE (10μm)	10 cm^2
9	Glass filled PTFE	30 cm^2
10*	None	None

The samples were centrifuged to harvest, then a Soxhlet extraction was performed and the resulting lipid dried under vacuum, whereupon acid transesterification was undertaken and the organic layer separated and dried for analysis by GCMS/TCD/FID.

4.15 References

1. Knothe, G., Monitoring a progressing transesterification reaction by fiber-optic near infrared spectroscopy with correlation to H^1 nuclear magnetic resonance spectroscopy. *J. Am. Oil Chem. Soc.* **2000,** *77* (5), 489-493.
2. Lubrizol Advanced Materials, Inc., Standard Operating Procedure Test Method for the Determination of Acid Value and Free Fatty Acid. Scher, T. P., Ed. 2005.

5 Further work

Varied avenues have been pursued throughout this work leading to conclusions which can be applied to future work. The use of pulsed LED bi-chromatic illumination could be further explored, particularly the ratio of the intensity of red to blue light and that of pulse width and pulse length to elucidate whether 10 ms is the reaction time for photosystem II reactions. Further work should ensure that nutrients are supplied in abundance for optimising lipid output, with regard to the FAME profiles obtained under the different nutrient conditions reported in this thesis. In the future using microwave extraction and transesterification as the standard method will allow comparable data for experiments to be obtained more quickly than using the Soxhlet and subsequent acid transesterification step.

Appendix 1

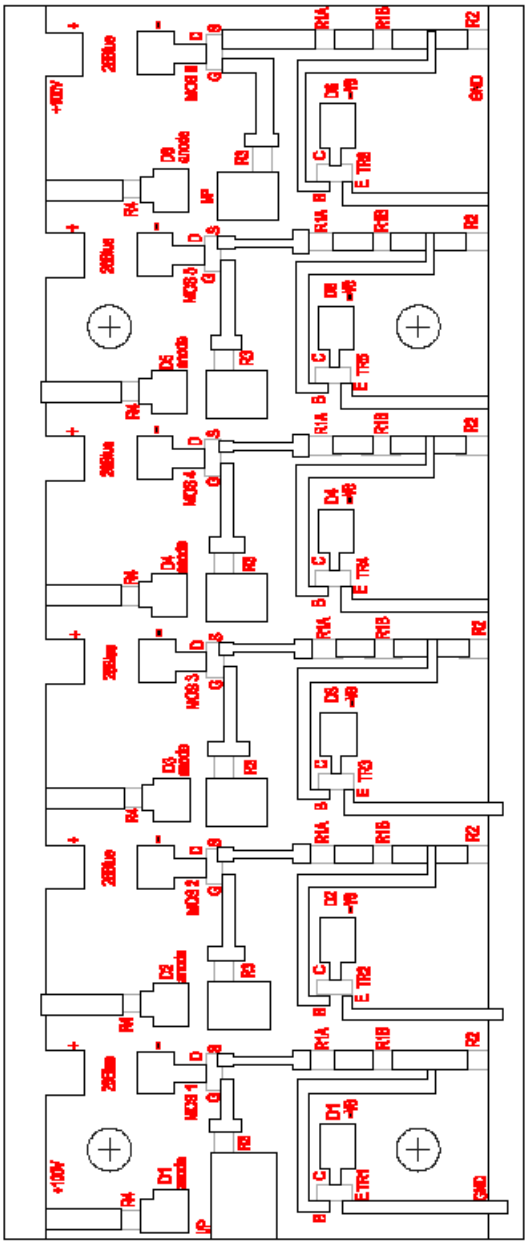


Figure 1 - Printed circuit board design

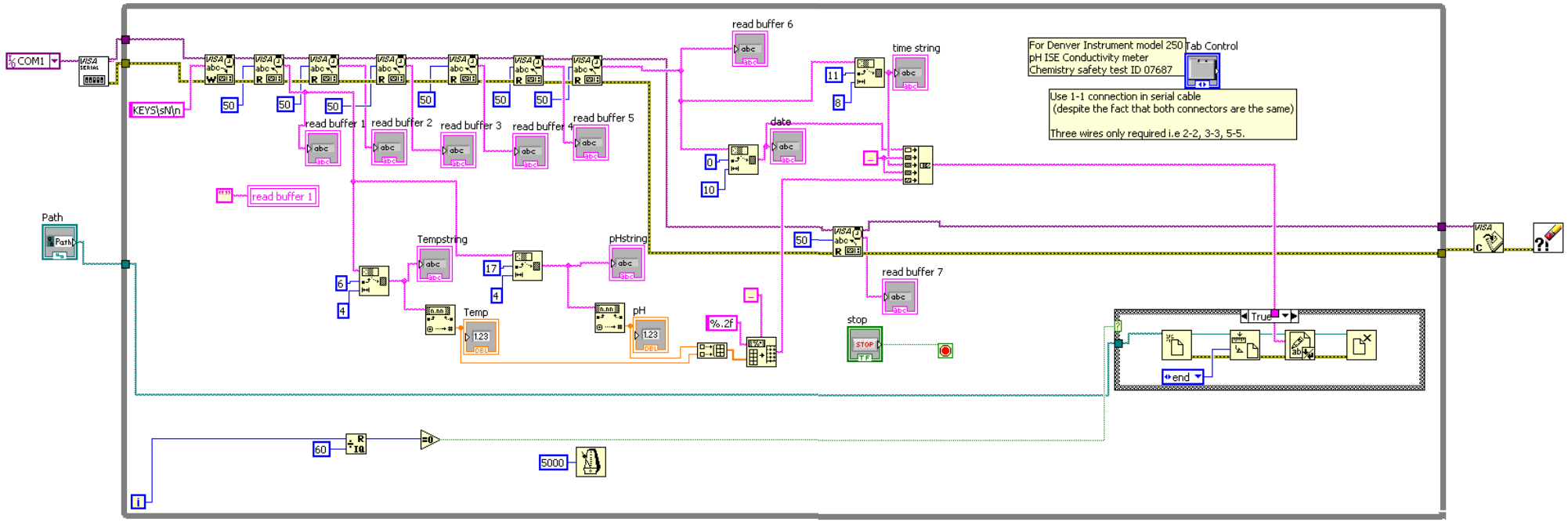


Figure 2 – Lab View 2010 temperature and pH logger.vi – block diagram

Table 1 - Algae biofuel companies; accessed May 2011

Company	Location	Business Aim	Reference
A2BE Carbon Capture, LLC	Colorado, USA	Use of algae for carbon capture and recycling.	www.algaeatwork.com
Alg Western Oil (OysseyOil)	Boshoek, South Africa	Use of algae biodiesel production to reduce carbon dioxide.	www.algbf.co.za
AlgaFuel S.A.	Olhão, Portugal	Use of bioengineering for the combined mitigation of CO_2 and production of biodiesel.	www.algafuel.pt
AlgaLink	Yerseke, The Netherlands	Design and manufacture of algae growing equipment and cultivation of algae for global markets	www.algaelink.com
Algenol Biofuels	Florida, USA	Use of patented algae technology to produce ethanol and development of photobioreactors	www.algenol.com
Allied Minds, Inc.	Boston, Massachusetts, USA	Production of biofuel from algae with minimal impact on environment	www.alliedminds.com
Aqua Solutions Biotech	Andalucia, Spain	Use of algae for non-fuel products	www.aquasolutionsbiotech.es
Aquaflow Bionomics	Marlborough, New Zealand	Use of algae for sewerage treatment and biodiesel production	www.aquaflowgroup.com
Aurora	Hayward, California, USA	Use of algae grown in seawater filled open ponds	www.aurorainc.com
Bioalgal Marine	Almeria, Spain	Use of intensive cattle farming and organic residues of agriculture to grow algae for fertiliser, cosmetics and pharmacological compounds	www.bioalgal.com
Biofuel Systems	Alicante, Spain	Use of waste CO_2 emissions to create oil.	www.biopetroleo.com
BioMara	Scotland	Government funded investigations into quick growing algae saline species	www.biomara.org
Bionavitas	Redmond, Washington, USA	Growth of algae for use as biofuels, neutraceuticals and environmental remediation.	www.bionavitas.com
Biotecnologia de microalgas, SL	Cádiz, Spain	Use of industrial microalgal production for fuel and cosmetic applications	www.andaluciabioregion.es/en/entity.cfm?eid=48
Blue Marble Energy	Seattle, USA	Use of waste flue gas grown algae to produce chemicals for plastics, food flavouring, fragrance and adhesives.	www.bluemarbleenergy.net

Company	Location	Business Aim	Reference
Bodega	MIT, Massachusetts, USA	Development of scalable algae photobioreactors.	www.bodegaalgae.com
Cellana (and HR BioPetroleum)	Kona, Hawaii, USA	Growth of algae in open ponds and bioreactors for use in the food and fuel markets.	www.cellana.com
CP Kelco	San Diego, USA	Use of macroalgae products for food additives.	www.cpkelco.com
Eni	Italy	Use of microalgae for cleaning of industrial wastewater and saline water and absorption of CO_2 from flue gas and the production of biodiesel.	www.eni.com
Global Green Solutions Inc.	Nevada, USA	Use of algae for preparation of low emission fuel	www.globalgreensolutionsinc.com
Greon	Bulgaria	Use of exhaust gases, heat, steam and other waste to produce biofuel	www.greon.eu
Ingrepro B.V.	Borculo, The Netherlands	Use of industrial algae production for nutritional and biomass purposes.	www.ingrepro.nl
Inventure	Alabama, USA	Use of algae for renewable energy	www.inventurechem.com
Kuehnle AgroSystems, Inc.	Honolulu and San Diego, USA	Use of a cross-disciplinary approach in developing algae seed-stocks and algal cultivators. Specialising in biofuels, carbon capture, speciality chemicals and neutraceuticals.	www.kuehnleagro.com
LGem B.V.	Voorhout, The Netherlands	Use of algae (*Nannochloropsis*) for fish food.	www.lgem.nl
Live Fuels	San Carlos, California, USA	Use of open ponds to grow algae, fish to harvest the algae and established technology to convert fish into renewable oils, fishmeal and high value dietary supplements.	www.livefuels.com/index.php
Necton S.A.	Olhão, Portugal	Use of algae growth and sea salt primarily for fish food.	www.necton.pt
Neste Oil	Helsinki, Finland	Use of algae alongside other renewable resources to provide fuel and lubricants	www.nesteoil.fi
Oil Fox	Capital Federal, Argentina	Use of algae for biodiesel and protein.	www.oilfox.com.ar
OriginOil S.A.	LA, California, USA	Development of the harvesting and dewatering stages in algal biodiesel production.	www.originoil.com
Petrosun	Arizona and Texas, USA	Use of micro and macroalgae for fuel, as finances allow.	www.petrosuninc.com
Phytonix Corporation	North Carolina, USA	Use of specific algae species grown under optimised conditions for development of at least 4 different types of biofuel with reduced greenhouse gas emissions.	www.phytonix.com

Company	Location	Business Aim	Reference
Sapphire Energy	California and New Mexico, USA	Use of algae to produce fuels including 91 octane gasoline, 89 cetane diesel, and jet fuel.	www.sapphireenergy.com
Seambiotic	Tel Aviv, Israel	Use of flue gas from coal burning power stations for algae growth. Production of biofuel and food additives.	www.seambiotic.com
SGC Energia SGPS, S.A.	Alhandra, Portugal	Use of algae for biofuel alongside other renewable energies	www.sgc.pt
Solazyme	San Francisco, California, USA	Use of heterotrophic growth of algae for fuel (biodiesel, diesel, ship diesel, jet diesel), chemicals, nutrition and health products	www.solazyme.com
Solena	Washington, USA	Algae gasification	www.solenagroup.com
Solix Biofuels	Colorado, USA	Use of closed algal bioreactors for biodiesel production	www.solixbiofuels.com
Sunrise Ridge Algae, Inc.	Katy, Texas, USA	Use of algae produced with minimal water and waste gases for fuel, and development of reactors and processing.	www.sunrise-ridge.com
Sur Algae	Cadiz, Spain	Use of algae for specialist food products	www.suralgae.com
Synthetic Genomics (and Exxon Mobil)	La Jolla, California, USA	Efficient and cost effective methods of producing biofuel from algae.	www.syntheticgenomics.com
Targetted Growth, Inc.	Seattle, Washington, USA	Use of algae for production of biodiesel, ethanol and jet fuel.	www.targetedgrowth.com
Wageningen University and Matalacanas	Wageningen, The Netherlands and Huelva, Spain	Use of seawater for algae research and pilot stage reactors	www.AlgaePARC.com
XL Renewables, Inc.	Arizona, USA	Use of large scale algae biomass production for tackling energy, food and environmental issues.	www.xldairygroup.com

Appendix 2

Table 2 - Toxicity test data (Figure 2-23)

	Material tested								
	plain 1	**plain 2**	**plain 3**	**neoprene 1**	**neoprene 2**	**neoprene 3**	**silicon grease**	**PTFE**	**glass filled PTFE**
Component	**ng in total sample**								
C14:0	0.00	32677.40	0.00	0.00	0.00	0.00	0.00	0.00	15345.10
C16:0	66153.80	2273805.00	457011.00	93616.70	150330.00	484831.00	908141.00	1456259.00	1503553.00
C16:1	8698.45	344827.00	54374.90	14475.90	25823.50	85347.20	137792.00	223144.00	175393.00
C16:2	3628.91	148525.00	25260.10	7248.00	12573.30	37370.50	58400.90	93862.30	107255.00
C16:3	26529.20	374906.00	67585.80	15043.70	23091.90	7930.68	118151.00	187821.00	230532.00
C16:4	0.00	653936.00	132481.00	30707.30	41206.60	165519.00	216304.00	296672.00	410531.00
C17:0	0.00	0.00	0.00	0.00	0.00	0.00	7107.01	12702.70	21669.90
C17:1	0.00	0.00	0.00	0.00	0.00	0.00	9095.59	0.00	0.00
C18:0	9308.54	245997.00	59318.20	9363.56	17908.10	49239.70	95048.30	162154.00	150120.00
C18:1n9c	125980.00	3240987.00	706626.00	143169.00	255036.00	744756.00	1221804.00	2078914.00	2076459.00
C18:2n6c	29331.90	827373.00	175680.00	35146.50	57647.20	182492.00	266450.00	428094.00	470036.00
C18:3n3	68247.20	2190270.00	422765.00	93908.90	146871.00	498287.00	678821.00	1004120.00	1301933.00
C18:4	11121.20	350906.00	70767.20	14678.00	20292.50	76487.60	96790.70	143530.00	200359.00
C20:0	0.00	17044.40	4373.58	0.00	0.00	0.00	5356.68	9907.93	8792.04
C20:1	0.00	199490.00	86711.40	0.00	0.00	22016.50	48337.90	93281.30	114879.00
TOTAL	**349000.00**	**10900746.00**	**2262953.00**	**457357.00**	**750779.00**	**2354276.00**	**3867602.00**	**6190462.00**	**6786856.00**

Appendix 3

Table 3 – Vertical configuration: Run 1: blue and red data (Figure 2-27)

Day	Date	Time	Time passed (Hr)	pH	CO2 (sl/min)	Air (sl/min)	Absorption	cell count (ml^-1)	cell count (M ml^-1)
0	15-Feb	11:00	0	6	0.213	0.403	0.075	375000	0.375
1	16-Feb	14:53	27.53	5.88	0.207	0.39	0.065	190000	0.190
2	17-Feb	10:20	47.2	5.46	0.207	0.39	0.058	310000	0.310
	17-Feb	15:16	52.16	6.22	0.208	0.403	0.122	420000	0.420
3	18-Feb	13:30	74.3	5.12	0.199	0.408	N/A	382500	0.383
5	20-Feb	08:36	117.39	5.05	0.196	0.424	0.1245	1682500	1.683
	20-Feb	15:31	124.31	5.96	0.208	0.387	0.134	1122500	1.123
	20-Feb	17:18	126.18	5.23	0.208	0.389	0.136	1007500	1.008
6	21-Feb	18:00	151		0.205	0.387		1000000	1.000
7	22-Feb	13:22	170.22		0.208	0.39	0.381	2756250	2.756
	22-Feb	17:31	174.31		0.207	0.403	0.44	2780000	2.780
8	23-Feb	12:00	193		0.205	0.39		3600000	3.600
	23-Feb	17:15	198.15		0.203	0.402		5420000	5.420
9	24-Feb	18:00	223	5.85	0.204	0.395		7695000	7.695
11	26-Feb	10:33	263.33		0.204	0.307		6656666	6.657
	26-Feb	16:22	269		0.201	0.307		6545000	6.545
13	28-Feb	09:05	310.05	6.19	0.201	0.385		11736666.7	11.737
	28-Feb	16:41	317.41	6.04	0.199	0.345		13445000	13.445
14	29-Feb	09:09	334.09		0.199	0.376		12900000	12.900

Table 4 - Run 2: Vertical configuration: blue and 1/12th red data (Figure 2-27)

Day	Date	Time	Time passed (Hr)	pH	CO2 (sl/min)	Air (sl/min)	Absorption	cell count (ml^-1)	cell count (M ml^-1)
0	29-Feb	11:05	0		0.203	0.379	0.022	132000	0.132
1	01-Mar	09:16	22.11		0.201	0.387	0.031	146900	0.147
2	02-Mar	10:02	46.57		0.202	0.384	0.037	147500	0.148
4	04-Mar	16:00	100.55		0.203	0.345	0.044	307500	0.308
5	05-Mar	12:01	120.56	4.45	0.201	0.39	0.123	450000	0.450
	05-Mar	15:52	124.47	4.53	0.204	0.386	0.094	565000	0.565
6	06-Mar	10:02	142.57		0.198	0.375	0.154	760000	0.760
	06-Mar	16:31	149.26		0.201	0.39	0.199	834500	0.835
7	07-Mar	09:49	166.44	4.8	0.199	0.328	0.254	1085000	1.085
8	08-Mar	10:17	191.12	5.6	0.203	0.384	0.483	1810000	1.810
9	09-Mar	19:00	223.55	6.57	0.2	0.378		2475000	2.475
10	10-Mar	17:00	245.55	6.57	0.2	0.387		3637500	3.638
12	12-Mar	09:30	286.25		0.199	0.376		3900000	3.900
	12-Mar	16:39	293.34		0.201	0.375		3875000	3.875
13	13-Mar	08:52	309.47		0.203	0.376	0.806	2825000	2.825
	13-Mar	16:36	317.31					3262500	3.263
14	14-Mar	09:14	334.09					2282500	2.283

Table 5 – Vertical configuration: Run 3 blue data (Figure 2-27)

Day	Date	Time	Time passed (Hr)	cell count (ml^-1)	cell count (M ml^-1)
0	14-Mar	10:28	0	30000	0.03
2	16-Mar	11:37	49.09	180000	0.18
		15:28	53	202500	0.2025
5	19-Mar	09:30	119.02	725000	0.725
		16:39	126.11	892500	0.8925
6	20-Mar	09:46	143.18	1787500	1.7875
		17:00	150.32	1747500	1.7475
7	21-Mar	10:26	167.58	2025000	2.025
		16:06	173.38	2085000	2.085
8	22-Mar	09:45	191.17	1620000	1.62
		16:57	198.29	1850000	1.85
9	23-Mar	13:05	218.37	2185000	2.185
10	24-Mar	17:00	246.32	3175000	3.175
12	26-Mar	12.29	290.01	2930000	2.93
13	27-Mar	10.39	312.11	2155000	2.155
		17:04	318.36	3437500	3.4375
14	28-Mar	10.25	335.57	2712500	2.7125

Run 4: Red light : 28th March – 11th April 2012: No data/no growth

Table 6 – Vertical configuration: Run 5: white data (Figure 2-27)

Day	Date	Time passed (Hr)	cell count (ml^-1)	cell count (M ml^-1)
0	28-Apr	0	14407	0.014407
1	29-Apr	24	27477	0.027477
2	30-Apr	48	62906	0.062906
3	01-May	72	158808	0.158808
4	02-May	96	460955	0.460955
9	11-May	168	5580540	5.58054
10	12-May	192	7447280	7.44728
11	13-May	216	8601150	8.60115
12	14-May	240	10490000	10.49
13	15-May	264	10519750	10.51975
14	16-May	336	10078400	10.0784

Table 7 – Light intensity and wavelength effect on algal growth data (Figure 2-28)

	Cell count (cells/ml)	**Light Reading Average (mmol)**
Continuous white light	10078400	80
Red and blue light (2: 1) pulsed	5664550	72.76
Red and blue light (2: 1) pulsed – repeat	12900000	72.76
Blue pulsed light	2712500	37.13
Red pulsed light (duplicated)	0	44.84

Table 8 – Cell vitality: *Chlorella emersonii* cell growth under standard conditions: white light, 20 ^{o}C, Bold's Basal solution, 3 % CO_2 (Figure 2-29)

Days after inoculation	**Non -viable cells / x10^6 cells/ml**	**Viable cells / x10^6 cells/ml**
0	0.049950	0.009514
1	0.035000	0.014407
2	0	0.027477
3	-	0.062906
4	-	0.158808
5	0.115000	0.460955
6	0.075000	-
8	-	5.580540
9	-	7.447280
10	-	8.601150
11	-	10.490000
12	-	10.519750
15	-	10.078400
40	3.958360	-

Table 9 - FAME component data for vertical reactor runs under differing light conditions (Figure 2-30)

	red and blue 2 weeks	red then red&blue 2 weeks each	blue 2 weeks	blue &1/12th red 2 weeks	continuous white light
Component	component / ng	component / ng	component / ng	component / ng	component / ng
Lauric acid methyl ester (C12:0)	0.00	0.00	1.12	0.00	
Myristic acid methyl ester (C14:0)	0.00	0.31	1.23	0.00	
Pentadecanoic acid methyl ester (C15:0)	0.00	0.45	0.59	0.00	
Palmitic acid methyl ester (C16:0)	37.34	14.51	52.32	42.66	32.60
Palmitoleic acid methyl ester (C16:1)	10.66	2.55	41.88	16.98	1.12
9,12-Hexadecadienoic acid, methyl ester (C16:2)	1.03	0.78	10.82	8.06	5.56
Hiragonic acid methyl ester (C16:3)	0.92	2.74	22.08	13.66	13.68
4,7,10,13-Hexadecatetraenoic acid, methyl ester, (4Z,7Z,10Z,13Z)- (C16:4)	40.99	11.94	60.58	47.14	
Heptadecanoic acid methyl ester (C17:0)	5.86	0.33	0.52	0.44	
cis-10-Heptadecanoic acid methyl ester (C17:1)	1.22	2.00	0.00	0.00	
Stearic acid methyl ester (C18:0)	0.65	0.39	0.92	0.66	3.38
Oleic acid methyl ester (C18:1n9c)	38.68	2.84	54.00	42.44	50.77
Linoleic acid methyl ester (C18:2n6c)	5.73	7.96	22.96	20.64	16.62
γ-Linolenic acid methyl ester (C18:3n6)	2.40	1.04	0.00	1.13	
Linolenic acid methyl ester (C18:3n3)	76.33	27.00	94.79	69.58	33.94
Stearidonic acid methyl ester (C18:4)	25.54	4.56	16.77	9.33	
cis-11-Eicosenoic acid methyl ester (C20:1)	0.40	0.50	0.85	0.00	

Appendix 4

Table 1 - *Chlorella emersonii* GC-MS results and subsequent calculations (Figure 3-3)

amount of dioxane used to dissolve sample / ul	2000.00						
	high calibration 6.71 mg/ml	a					
Component	**weight of component / ng**	**average integration value**	**FID area**	**% biodiesel**	**component / ng**	**ng in total algae sample**	**grammes in total sample**
Butyric acid methyl ester (C4:0)	8.13						
Caproic acid methyl ester (C6:0)	8.13						
Caprylic acid methyl ester (C8:0)	8.13	8043548.60		0.00	0.00	0.00	0.000000000
Capric acid methyl ester (C10:0)	8.13	8043548.60		0.00	0.00	0.00	0.000000000
Undecanoic acid methyl ester (C11:0)	4.07	4176858.80		0.00	0.00	0.00	0.000000000
Lauric acid methyl ester (C12:0)	8.13	7949810.60		0.00	0.00	0.00	0.000000000
Tridecanoic acid methyl ester (C13:0)	4.07	3934779.20		0.00	0.00	0.00	0.000000000
Myristic acid methyl ester (C14:0)	8.13	8086004.20		0.00	0.00	0.00	0.000000000
Myristoleic acid methyl ester (C14:1)	4.07	4178624.80		0.00	0.00	0.00	0.000000000
Pentadecanoic acid methyl ester (C15:0)	4.07	3997595.40		0.00	0.00	0.00	0.000000000
cis-10-Pentadecenoic acid methyl ester (C15:1)	4.07	3919311.80		0.00	0.00	0.00	0.000000000
Palmitic acid methyl ester (C16:0)	12.20	12487814.40	33370950.00	2.67	32.60	2151720.72	0.002151721
Palmitoleic acid methyl ester (C16:1)	4.07	3991272.20	1101713.00	0.28	1.12	74147.32	0.000074147

Component	weight of component / ng	average integration value	FID area	% biodiesel	component / ng	ng in total algae sample	grammes in total sample
9,12-Hexadecadienoic acid, methyl ester (C16:2)	4.07	4444501.12	5229232.00	1.37	5.56	366914.97	0.000366915
Hiragonic acid methyl ester (C16:3)	4.07	4444501.12	12864292.00	3.36	13.68	902637.59	0.000902638
4,7,10,13-Hexadecatetraenoic acid, methyl ester, (4Z,7Z,10Z,13Z)- (C16:4)	4.07	4444501.12		0.00	0.00	0.00	0.000000000
Heptadecanoic acid methyl ester (C17:0)	4.07	4036219.40		0.00	0.00	0.00	0.000000000
cis-10-Heptadecanoic acid methyl ester (C17:1)	4.07	4413445.20		0.00	0.00	0.00	0.000000000
Stearic acid methyl ester (C18:0)	8.13	7999467.00	3323544.00	0.42	3.38	222933.26	0.000222933
Elaidic acid methyl ester (C18:1n9t)	4.07	4475340.20		0.00	0.00	0.00	0.000000000
Oleic acid methyl ester (C18:1n9c)	8.13	9382559.00	58589614.00	6.24	50.77	3350686.64	0.003350687
Linolelaidic acid methyl ester (C18:2n6t)	4.07	4377734.20		0.00	0.00	0.00	0.000000000
Linoleic acid methyl ester (C18:2n6c)	4.07	4304864.80	17575362.00	4.08	16.62	1096688.04	0.001096688
γ-Linolenic acid methyl ester (C18:3n6)	8.13	3958868.40		0.00	0.00	0.00	0.000000000
Linolenic acid methyl ester (C18:3n3)	4.07	3946498.20	32908429.00	8.34	33.94	2239925.56	0.002239926
Stearidonic acid methyl ester (C18:4)	4.07	4444501.12		0.00	0.00	0.00	0.000000000
Arachidic acid methyl ester (C20:0)	4.07	8011965.20		0.00	0.00	0.00	0.000000000
cis-11-Eicosenoic acid methyl ester (C20:1)	4.07	4538155.60		0.00	0.00	0.00	0.000000000
cis-11,14-Eicosenoic acid methyl ester (C20:2)	4.07	3600012.40		0.00	0.00	0.00	0.000000000

Component	weight of component / ng	average integration value	FID area	% biodiesel	component / ng	ng in total algae sample	grammes in total sample
Heneicosanoic acid methyl ester (C21:0)	4.07	4112130.40		0.00	0.00	0.00	0.000000000
cis-8,11,14-Eicosatrienoic acid methyl ester (C20:3n6)	8.13	4308608.20		0.00	0.00	0.00	0.000000000
Arachidonic acid methyl ester (C20:4n6)	4.07	3747175.20		0.00	0.00	0.00	0.000000000
cis-11,14,17-Eicosatrienoic acid methyl ester (C20:3n3)	4.07	4119730.40		0.00	0.00	0.00	0.000000000
Behenic acid methyl ester (C22:0)	4.07	6628534.60		0.00	0.00	0.00	0.000000000
cis-5,8,11,14,17-Eicosapentaenoic acid methyl ester (C20:5n3)	4.07	4859522.00		0.00	0.00	0.00	0.000000000
Erucic acid methyl ester (C22:1n9)	4.07	4460507.60		0.00	0.00	0.00	0.000000000
cis-13,16-Docosadienoic acid methyl ester (C22:2)	4.07	2953653.40		0.00	0.00	0.00	0.000000000
Tricosanoic acid methyl ester (C23:0)	8.13	4596000.40		0.00	0.00	0.00	0.000000000
Lignoceric acid methyl ester (C24:0)	4.07	7084519.80		0.00	0.00	0.00	0.000000000
Nervonic acid methyl ester (C24:1)	4.07	3415734.40		0.00	0.00	0.00	0.000000000
cis-4,7,10,13,16,19-Docosahexaenoic acid methyl ester (C22:6n3)	4.07	3828342.80		0.00	0.00	0.00	0.000000000
TOTAL	**203.38**	**177925208.80**					

Explanation of GC-MS calculations

The sample was injected onto the GC column via the injection chamber. The injection chamber discarded a set amount of the sample to waste and injected the remainder of the sample into the GC column. In this case the split ratio was set to 10:1 i.e. 10 parts waste to 1 part entered the column for processing. The sample eluted through the GC column and entered the splitter plate, where it was split further into three equal parts and each third headed to one of the three detectors; MS, FID, TCD.

The MS results were used to identify each peak in the sample, i.e. for qualification. The FID was used to quantify the peaks within the sample, therefore a known concentration of each peak and its peak integration was elucidated using calibrant samples. The calibrant used for all calculations within this thesis was FAME mix C4-C24 *Supleco* (18919-1AMP) at a concentration of 6.71 mg ml^{-1}. The 3 calibrant samples were run in sets of 5 to ensure repeatability and reliability; 3 different concentrations were run to confirm that peak integration related consistently to sample concentration (section 4.8).

Using the known weights (from the supplied data sheet of the standard) of the separate components of the standard FAMEs , the FID responses per unit mass of the separate calibrant components were recorded in column C (different molecules have different responses). In column B the calculated amount of each FAME which should be detected at the FID was recorded. These two columns allowed for the responses of unknown samples to be calculated into weights and percentages.

For a sample each FAME peak was first checked on the MS to allow for qualification of the identity of the peak and then corresponding peak on the FID was integrated. The integrated reading/response on the FID was compared to the average calibrant response, the ratio was taken of the sample integration/average calibrant integration for the relevant FAME to give the "% biodiesel" as shown in column E. In column F, "component/ng", this value was multiplied by the mass of the calibrant FAME which determined the amount of the specific FAME in the sample which is being detected at the FID.

The sample was known to have been split into 3 at the splitter plate and previously split into 11 therefore the sample required multiplying by 3 and 11 (or by 33) and then also multiplying by the amount of dioxane the total sample was dissolved in – in µl (to give a reading for the entire biodiesel sample – in this case 2000 µl) hence column G which took into account all three of these splittings.

Since column G was in nanogrammes the final column converts the amounts back to grammes, which may or may not be more useful for the sample size used (for the amounts of algae and thus biodiesel yielded in these experiments ng is more useful).

Appendix 5

Table 2 - FAME components by percentage for terrestrial biodiesel sources and *Chlorella emersonii* biodiesel (Figure 3-2)

FAME components / %	**Coconut biodiesel**	**Rapeseed biodiesel**	**Palm oil biodiesel**	**Waste oil biodiesel**	***Chlorella emersonii* biodiesel**
Caprylic acid methyl ester (C8:0)	6.8				
Capric acid methyl ester (C10:0)	5.7				
Lauric acid methyl ester (C12:0)	46.4				
Myristic acid methyl ester (C14:0)	18.6		0.9		
Palmitic acid methyl ester (C16:0)	6.0	4.7	42.8	15.9	10.0
Palmitoleic acid methyl ester (C16:1)	3.8	1.5			1.0
9,12-Hexadecadienoic acid, methyl ester (C16:2)					5.1
Hiragonic acid methyl ester (C16:3)					12.6
Stearic acid methyl ester (C18:0)	3.2	61.3	4.5	3.5	1.6
Oleic acid methyl ester (C18:1n9c)	7.2	20.1	40.7	26.9	23.3
Linoleic acid methyl ester (C18:2n6c)	2.0		10.2	47.1	15.3
Linolenic acid methyl ester (C18:3n3)		9.6		5.5	31.2
cis-11-Eicosenoic acid methyl ester (C20:1)		1.3			
Total / %	**99.8**	**98.5**	**99.0**	**98.9**	**100.0**

Table 3 – Culture growth for Box-Behnken *Chlorella emersonii* inoculation culture (Figure 3-5)

Days after inoculation	Cell count of inoculation culture (million cells / ml)
0	0.07
1	0.21
2	0.60
3	0.65
4	4.00
5	
6	13.05
7	17.60
8	30.10
9	4.55
10	3.75

Appendix 6

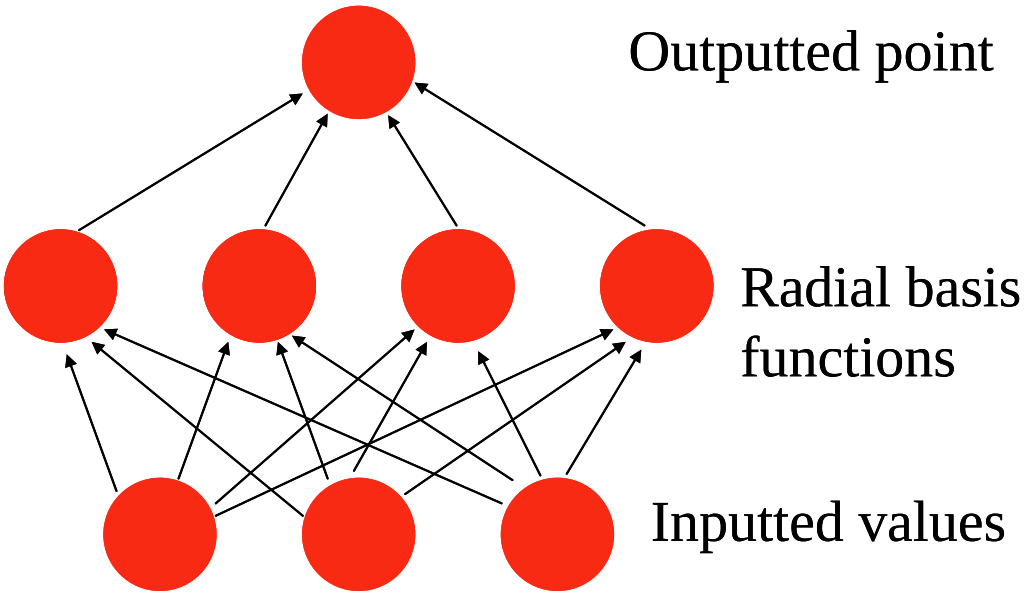


Figure 1 - Schematic for radial basis function networks for data point determination and validity

Each point on the 3-D graphs produced from the MATLAB software was determined using the input data, 3 values for each point. These three values were combined to produce 4 values (Figure 1) and all of these values were used to produce each outputted data value on the 3-D graph and in Tables 13 – 83. The validity of these outputted values was determined by recalculating the outputted point four times, each time with one of the radial basis functions removed. The variation in the outputted

point was desired to be low for each of these calculations as this would suggest that the data point prediction was very accurate.

The prediction capabilities of the Box-Behnken design were checked for each of the outputs by the iterative graph shown below (Figure 2). As can be seen the actual percentage of saturated FAMEs was plotted along the x axis against the predicted values on the y axis. There were 27 points for each of the tests, 24 with varying factors and 3 repeated control experiments. The closeness of fit to the trend line shows how accurate the statistical data was for this prediction. As this has a close fit in this case (Figure 2), predictions can be made with confidence for the effect of changing factors on the percentage of, in this case, saturated FAMEs produced from the lipids of *Chlorella emersonii* grown in specific conditions.

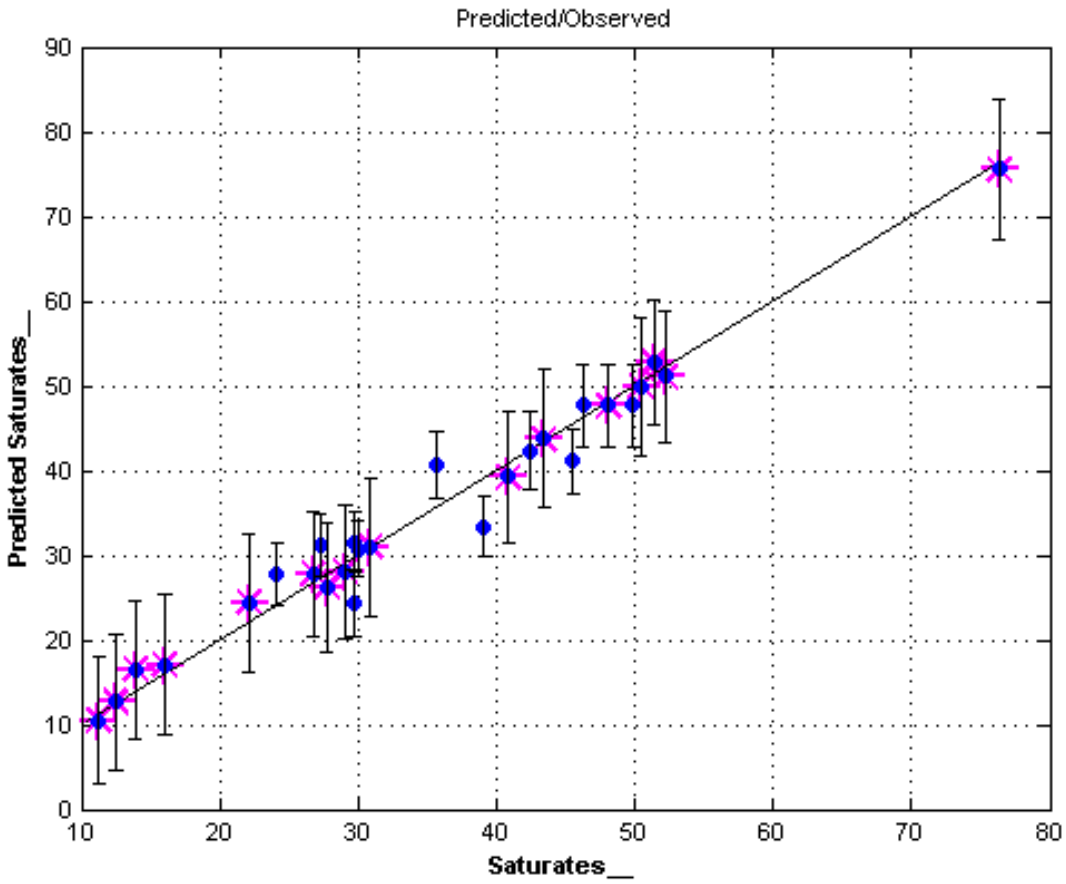


Figure 2 - Predicted outcome of total percentage of saturated FAMEs compared to that of the observed total percentage of saturated FAMEs for all experiments

Appendix 7

Table 4 - Radial basis functions determined by *MATLAB*® for C18(1) nitrates vs iron; where nitrates amount 5 means 0.125 g/l, 10 = 0.250 g/l and 15 = 0.375g/l; where iron amount 5 means 2.49 mg/l, 10 = 4.98 mg/l and 15 = 7.47 mg/l (Figure 3-6a)

C18(1) / %	Nitrates																				
Iron	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
5	33.69	32.49	31.34	30.25	29.25	28.35	27.57	26.94	26.47	26.17	26.07	26.16	26.43	26.89	27.52	28.29	29.21	30.23	31.36	32.56	33.82
5.5	33.26	32.04	30.87	29.76	28.73	27.80	27.01	26.36	25.88	25.58	25.47	25.57	25.86	26.33	26.98	27.79	28.73	29.79	30.94	32.17	33.46
6	32.89	31.66	30.47	29.34	28.29	27.35	26.53	25.87	25.38	25.08	24.98	25.08	25.39	25.88	26.55	27.38	28.35	29.44	30.62	31.88	33.19
6.5	32.57	31.33	30.13	28.98	27.92	26.96	26.14	25.47	24.98	24.68	24.58	24.69	25.01	25.52	26.22	27.07	28.07	29.18	30.39	31.67	33.00
7	32.30	31.05	29.84	28.68	27.61	26.65	25.82	25.15	24.65	24.36	24.27	24.40	24.73	25.26	25.98	26.85	27.88	29.01	30.24	31.54	32.89
7.5	32.04	30.79	29.58	28.42	27.35	26.38	25.55	24.89	24.40	24.11	24.04	24.18	24.53	25.08	25.81	26.72	27.76	28.92	30.17	31.49	32.86
8	31.80	30.55	29.34	28.19	27.12	26.16	25.34	24.68	24.20	23.93	23.87	24.03	24.40	24.97	25.73	26.65	27.72	28.90	30.17	31.50	32.88
8.5	31.54	30.30	29.10	27.96	26.90	25.96	25.15	24.51	24.05	23.80	23.76	23.94	24.33	24.93	25.71	26.65	27.74	28.93	30.22	31.56	32.95
9	31.25	30.03	28.85	27.72	26.69	25.77	24.98	24.37	23.94	23.72	23.70	23.91	24.32	24.94	25.74	26.70	27.81	29.02	30.32	31.67	33.06
9.5	30.92	29.72	28.56	27.46	26.46	25.57	24.83	24.25	23.86	23.67	23.69	23.92	24.37	25.01	25.83	26.81	27.92	29.15	30.45	31.81	33.21
10	30.52	29.35	28.22	27.17	26.21	25.37	24.68	24.15	23.81	23.66	23.72	23.98	24.46	25.12	25.96	26.95	28.08	29.31	30.62	31.98	33.38
10.5	30.07	28.92	27.84	26.84	25.94	25.16	24.53	24.06	23.78	23.68	23.78	24.09	24.59	25.28	26.13	27.14	28.27	29.51	30.82	32.18	33.57
11	29.54	28.43	27.40	26.47	25.64	24.94	24.39	23.99	23.77	23.74	23.89	24.24	24.77	25.48	26.35	27.37	28.50	29.74	31.04	32.40	33.78
11.5	28.95	27.89	26.92	26.06	25.33	24.72	24.26	23.95	23.80	23.83	24.04	24.43	24.99	25.73	26.61	27.63	28.77	30.00	31.29	32.64	34.01
12	28.31	27.29	26.40	25.63	25.00	24.51	24.15	23.93	23.87	23.97	24.24	24.67	25.27	26.02	26.92	27.94	29.08	30.30	31.58	32.91	34.28
12.5	27.62	26.65	25.85	25.20	24.70	24.32	24.07	23.96	23.99	24.16	24.49	24.97	25.60	26.37	27.28	28.30	29.43	30.64	31.91	33.23	34.57
13	26.89	25.99	25.30	24.79	24.43	24.19	24.06	24.06	24.17	24.42	24.81	25.33	25.99	26.78	27.69	28.71	29.83	31.02	32.28	33.58	34.91
13.5	26.14	25.32	24.78	24.44	24.23	24.14	24.13	24.23	24.44	24.76	25.20	25.76	26.45	27.25	28.17	29.18	30.29	31.47	32.70	33.98	35.29
14	25.38	24.68	24.34	24.20	24.17	24.21	24.32	24.52	24.80	25.18	25.67	26.27	26.97	27.79	28.70	29.71	30.80	31.96	33.18	34.44	35.73
14.5	24.60	24.14	24.10	24.17	24.28	24.44	24.65	24.93	25.27	25.71	26.23	26.85	27.58	28.40	29.31	30.31	31.38	32.52	33.71	34.95	36.22
15	23.83	24.04	24.24	24.43	24.64	24.88	25.15	25.47	25.87	26.34	26.89	27.53	28.26	29.08	29.98	30.97	32.02	33.14	34.31	35.52	36.77

Table 5 - Radial basis functions determined by *MATLAB*® for C18(1) nitrates vs phosphates; where nitrates amount 5 means 0.125 g/l, 10 = 0.250 g/l and 15 = 0.375g/l; where phosphate amount 5 means a ratio of K_2HPO_4:KH_2PO_4 of 0.0375:0.0875 g/l, 10 = 0.0750:0.1750 g/l and 15 = 0.1125:0.2625 g/l (Figure 3-6b)

C18(1) / %	Nitrates																				
Phosphates	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
5	31.53	30.39	29.31	28.31	27.40	26.61	25.96	25.45	25.12	24.96	25.00	25.23	25.65	26.24	27.00	27.91	28.94	30.08	31.30	32.58	33.92
5.5	31.16	30.01	28.91	27.90	26.98	26.17	25.50	24.99	24.65	24.49	24.53	24.76	25.19	25.80	26.58	27.51	28.56	29.72	30.96	32.27	33.62
6	30.88	29.71	28.61	27.58	26.64	25.83	25.15	24.63	24.28	24.13	24.17	24.41	24.85	25.47	26.26	27.21	28.28	29.45	30.71	32.04	33.40
6.5	30.67	29.50	28.38	27.35	26.40	25.58	24.89	24.37	24.02	23.86	23.91	24.16	24.60	25.24	26.04	27.00	28.08	29.27	30.55	31.88	33.26
7	30.53	29.36	28.23	27.19	26.24	25.41	24.72	24.19	23.84	23.69	23.74	23.99	24.44	25.08	25.90	26.87	27.96	29.17	30.45	31.80	33.18
7.5	30.45	29.27	28.15	27.10	26.14	25.31	24.62	24.09	23.74	23.58	23.64	23.89	24.35	25.00	25.82	26.80	27.91	29.12	30.41	31.77	33.16
8	30.42	29.23	28.11	27.05	26.10	25.26	24.57	24.04	23.69	23.54	23.59	23.85	24.31	24.97	25.80	26.78	27.90	29.12	30.42	31.78	33.17
8.5	30.41	29.23	28.11	27.05	26.09	25.26	24.56	24.03	23.68	23.53	23.59	23.85	24.32	24.98	25.81	26.80	27.92	29.15	30.45	31.82	33.21
9	30.44	29.26	28.13	27.08	26.12	25.28	24.58	24.05	23.71	23.56	23.61	23.88	24.35	25.01	25.85	26.84	27.97	29.20	30.51	31.87	33.26
9.5	30.48	29.30	28.17	27.12	26.16	25.32	24.62	24.09	23.75	23.60	23.66	23.93	24.40	25.06	25.90	26.90	28.02	29.25	30.56	31.93	33.32
10	30.52	29.35	28.22	27.17	26.21	25.37	24.68	24.15	23.81	23.66	23.72	23.98	24.46	25.12	25.96	26.95	28.08	29.31	30.62	31.98	33.38
10.5	30.58	29.41	28.28	27.23	26.28	25.44	24.75	24.22	23.87	23.73	23.79	24.05	24.52	25.18	26.02	27.02	28.14	29.37	30.67	32.03	33.42
11	30.64	29.47	28.35	27.30	26.35	25.52	24.83	24.30	23.96	23.81	23.87	24.13	24.60	25.26	26.09	27.08	28.20	29.42	30.73	32.08	33.47
11.5	30.72	29.55	28.44	27.39	26.44	25.61	24.92	24.40	24.06	23.91	23.97	24.23	24.69	25.35	26.17	27.16	28.27	29.49	30.78	32.13	33.52
12	30.82	29.66	28.54	27.50	26.56	25.73	25.05	24.53	24.18	24.04	24.09	24.35	24.81	25.46	26.28	27.25	28.36	29.57	30.85	32.20	33.58
12.5	30.96	29.79	28.68	27.65	26.71	25.89	25.21	24.69	24.35	24.20	24.25	24.51	24.96	25.60	26.42	27.38	28.47	29.67	30.95	32.29	33.66
13	31.13	29.97	28.87	27.84	26.91	26.10	25.42	24.91	24.57	24.42	24.47	24.72	25.16	25.80	26.60	27.55	28.64	29.82	31.09	32.42	33.78
13.5	31.36	30.21	29.12	28.10	27.17	26.37	25.70	25.19	24.85	24.70	24.74	24.99	25.43	26.05	26.85	27.79	28.85	30.03	31.28	32.59	33.95
14	31.66	30.51	29.43	28.42	27.51	26.71	26.05	25.54	25.21	25.06	25.10	25.34	25.78	26.39	27.17	28.09	29.14	30.30	31.54	32.84	34.18
14.5	32.02	30.89	29.82	28.83	27.93	27.14	26.49	25.99	25.66	25.52	25.56	25.79	26.21	26.81	27.57	28.48	29.51	30.65	31.87	33.15	34.48
15	32.46	31.35	30.29	29.32	28.44	27.67	27.03	26.54	26.22	26.07	26.11	26.34	26.75	27.34	28.08	28.97	29.97	31.09	32.28	33.54	34.85

Table 6 - Radial basis functions determined by *MATLAB*® for C18(1) nitrates vs time; where nitrates amount 5 means 0.125 g/l, 10 = 0.250 g/l and 15 = 0.375g/l (Figure 3-6c)

C18(1) / %	**Nitrates**																				
Time / days	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
3	29.80	29.05	28.42	27.93	27.60	27.43	27.45	27.67	28.11	28.77	29.66	30.79	32.16	33.75	35.57	37.59	39.79	42.18	44.72	47.41	50.23
3.2	29.17	28.39	27.73	27.20	26.83	26.63	26.62	26.81	27.21	27.84	28.71	29.81	31.15	32.72	34.51	36.50	38.67	41.00	43.44	45.88	47.90
3.4	28.68	27.85	27.16	26.60	26.19	25.95	25.90	26.05	26.41	27.00	27.83	28.89	30.18	31.69	33.42	35.33	37.40	39.59	41.82	43.95	45.72
3.6	28.32	27.46	26.72	26.12	25.67	25.39	25.30	25.40	25.71	26.25	27.02	28.01	29.24	30.67	32.31	34.11	36.05	38.08	40.10	42.01	43.68
3.8	28.10	27.21	26.43	25.79	25.29	24.96	24.82	24.86	25.12	25.59	26.28	27.20	28.34	29.68	31.21	32.89	34.69	36.56	38.42	40.19	41.79
4	28.04	27.11	26.29	25.60	25.05	24.67	24.46	24.44	24.62	25.02	25.63	26.46	27.50	28.74	30.15	31.71	33.37	35.09	36.82	38.48	40.04
4.2	28.16	27.18	26.31	25.57	24.96	24.51	24.23	24.14	24.24	24.55	25.07	25.80	26.73	27.86	29.15	30.58	32.12	33.72	35.33	36.92	38.43
4.4	28.45	27.43	26.50	25.70	25.02	24.50	24.13	23.95	23.96	24.17	24.59	25.21	26.04	27.04	28.22	29.53	30.95	32.44	33.97	35.48	36.97
4.6	28.93	27.86	26.88	26.00	25.25	24.63	24.18	23.89	23.80	23.90	24.20	24.71	25.42	26.31	27.37	28.57	29.89	31.28	32.72	34.18	35.64
4.8	29.62	28.50	27.45	26.49	25.64	24.92	24.36	23.96	23.74	23.73	23.91	24.30	24.89	25.67	26.62	27.71	28.93	30.24	31.61	33.02	34.44
5	30.52	29.35	28.22	27.17	26.21	25.37	24.68	24.15	23.81	23.66	23.72	23.98	24.46	25.12	25.96	26.95	28.08	29.31	30.62	31.98	33.38
5.2	31.65	30.41	29.20	28.04	26.96	25.98	25.15	24.47	23.98	23.70	23.62	23.76	24.11	24.66	25.40	26.30	27.35	28.51	29.76	31.08	32.44
5.4	33.00	31.70	30.40	29.11	27.89	26.75	25.76	24.92	24.27	23.84	23.62	23.63	23.86	24.30	24.94	25.76	26.73	27.82	29.02	30.30	31.64
5.6	34.59	33.22	31.80	30.38	28.99	27.68	26.50	25.49	24.68	24.08	23.72	23.60	23.71	24.05	24.59	25.33	26.22	27.26	28.41	29.65	30.97
5.8	36.41	34.97	33.42	31.83	30.25	28.74	27.37	26.17	25.18	24.43	23.92	23.67	23.67	23.90	24.36	25.01	25.84	26.83	27.93	29.14	30.43
6	38.46	36.94	35.25	33.46	31.66	29.93	28.35	26.95	25.78	24.87	24.22	23.84	23.72	23.86	24.24	24.82	25.59	26.52	27.59	28.76	30.02
6.2	40.75	39.13	37.25	35.22	33.18	31.21	29.41	27.81	26.47	25.40	24.61	24.11	23.89	23.94	24.24	24.76	25.47	26.35	27.38	28.52	29.75
6.4	43.28	41.54	39.40	37.08	34.75	32.54	30.51	28.73	27.22	26.01	25.10	24.49	24.18	24.14	24.37	24.83	25.49	26.32	27.30	28.41	29.62
6.6	46.03	44.10	41.60	38.92	36.30	33.85	31.63	29.68	28.03	26.70	25.68	24.97	24.58	24.47	24.63	25.03	25.64	26.43	27.37	28.45	29.62
6.8	49.02	46.67	43.62	40.58	37.71	35.07	32.71	30.64	28.88	27.45	26.35	25.57	25.10	24.93	25.03	25.38	25.94	26.68	27.59	28.62	29.76
7	52.24	48.56	45.09	41.86	38.87	36.15	33.72	31.58	29.76	28.27	27.10	26.27	25.75	25.52	25.57	25.87	26.38	27.08	27.94	28.94	30.04

Table 7 – Radial basis functions determined by *MATLAB*® for C18(1) iron vs time; where iron amount 5 means 2.49 mg/l, 10 = 4.98 mg/l and 15 = 7.47 mg/l (Figure 3-6d)

C18(1) / %	Iron																				
Time / days	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
3	33.92	33.23	32.60	32.02	31.50	31.04	30.64	30.30	30.02	29.81	29.66	29.58	29.57	29.64	29.77	29.98	30.27	30.64	31.08	31.60	32.20
3.2	32.96	32.27	31.63	31.06	30.54	30.08	29.68	29.34	29.07	28.86	28.71	28.63	28.62	28.68	28.82	29.03	29.32	29.68	30.13	30.66	31.27
3.4	32.03	31.34	30.71	30.14	29.63	29.17	28.78	28.45	28.18	27.97	27.83	27.75	27.74	27.80	27.94	28.15	28.43	28.80	29.25	29.78	30.40
3.6	31.15	30.47	29.84	29.27	28.77	28.32	27.94	27.62	27.35	27.15	27.02	26.94	26.94	27.00	27.13	27.34	27.63	27.99	28.45	28.98	29.61
3.8	30.31	29.63	29.01	28.46	27.96	27.53	27.16	26.85	26.60	26.41	26.28	26.22	26.22	26.28	26.42	26.62	26.91	27.28	27.73	28.27	28.90
4	29.52	28.84	28.23	27.69	27.21	26.80	26.45	26.16	25.92	25.75	25.63	25.58	25.58	25.65	25.79	26.00	26.28	26.65	27.10	27.65	28.28
4.2	28.76	28.09	27.50	26.97	26.52	26.13	25.80	25.53	25.32	25.17	25.07	25.03	25.04	25.12	25.26	25.47	25.76	26.13	26.58	27.13	27.77
4.4	28.03	27.38	26.81	26.31	25.88	25.51	25.22	24.98	24.79	24.67	24.59	24.57	24.60	24.69	24.84	25.05	25.34	25.71	26.17	26.72	27.37
4.6	27.35	26.71	26.16	25.69	25.29	24.96	24.70	24.50	24.35	24.25	24.20	24.21	24.26	24.36	24.52	24.75	25.04	25.42	25.88	26.43	27.08
4.8	26.69	26.08	25.55	25.11	24.75	24.47	24.25	24.09	23.98	23.93	23.91	23.94	24.02	24.14	24.32	24.56	24.86	25.25	25.71	26.27	26.92
5	26.07	25.47	24.98	24.58	24.27	24.04	23.87	23.76	23.70	23.69	23.72	23.78	23.89	24.04	24.24	24.49	24.81	25.20	25.67	26.23	26.89
5.2	25.47	24.90	24.45	24.10	23.84	23.67	23.56	23.51	23.51	23.55	23.62	23.73	23.87	24.05	24.27	24.54	24.88	25.28	25.76	26.32	26.98
5.4	24.90	24.36	23.95	23.65	23.46	23.35	23.32	23.34	23.40	23.50	23.62	23.78	23.96	24.17	24.42	24.71	25.06	25.48	25.97	26.54	27.20
5.6	24.34	23.84	23.48	23.25	23.13	23.11	23.15	23.24	23.38	23.54	23.72	23.93	24.15	24.40	24.68	25.00	25.37	25.80	26.30	26.88	27.54
5.8	23.80	23.33	23.03	22.89	22.86	22.92	23.06	23.24	23.45	23.68	23.92	24.18	24.45	24.74	25.05	25.40	25.79	26.23	26.75	27.33	28.00
6	23.27	22.84	22.62	22.57	22.65	22.82	23.05	23.32	23.61	23.91	24.22	24.53	24.84	25.17	25.52	25.89	26.30	26.77	27.29	27.88	28.55
6.2	22.74	22.36	22.24	22.31	22.51	22.80	23.14	23.50	23.87	24.24	24.61	24.97	25.33	25.70	26.08	26.48	26.91	27.39	27.93	28.52	29.19
6.4	22.21	21.91	21.92	22.14	22.48	22.89	23.33	23.79	24.23	24.67	25.10	25.51	25.91	26.31	26.72	27.15	27.60	28.10	28.64	29.25	29.92
6.6	21.68	21.48	21.69	22.09	22.59	23.12	23.66	24.19	24.71	25.20	25.68	26.13	26.57	27.01	27.44	27.89	28.37	28.87	29.43	30.04	30.71
6.8	21.14	21.17	21.66	22.26	22.89	23.52	24.13	24.72	25.29	25.83	26.35	26.84	27.31	27.77	28.23	28.70	29.19	29.71	30.28	30.89	31.56
7	20.59	21.31	22.03	22.74	23.43	24.11	24.76	25.39	25.99	26.56	27.10	27.62	28.12	28.60	29.08	29.57	30.07	30.61	31.18	31.79	32.46

Table 8 - Radial basis functions determined by *MATLAB*® for C18(1) iron vs phosphates; where iron amount 5 means 2.49 mg/l, 10 = 4.98 mg/l and 15 = 7.47 mg/l; where phosphate amount 5 means a ratio of K_2HPO_4:KH_2PO_4 of 0.0375:0.0875 g/l, 10 = 0.0750:0.1750 g/l and 15 = 0.1125:0.2625 g/l (Figure 3-6e)

C18(1) / %	Iron																				
Phosphates	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
5	29.11	28.35	27.66	27.04	26.49	26.02	25.63	25.33	25.12	25.01	25.00	25.09	25.28	25.56	25.93	26.39	26.93	27.54	28.23	28.98	29.80
5.5	28.49	27.74	27.06	26.46	25.94	25.49	25.12	24.83	24.64	24.54	24.53	24.62	24.80	25.07	25.43	25.87	26.39	26.99	27.65	28.39	29.19
6	27.93	27.21	26.55	25.97	25.47	25.05	24.71	24.44	24.26	24.17	24.17	24.25	24.43	24.68	25.03	25.45	25.94	26.51	27.16	27.87	28.66
6.5	27.46	26.75	26.12	25.57	25.10	24.70	24.39	24.15	23.99	23.91	23.91	23.99	24.15	24.40	24.71	25.11	25.58	26.12	26.74	27.43	28.20
7	27.05	26.36	25.76	25.24	24.80	24.43	24.15	23.94	23.80	23.73	23.74	23.82	23.97	24.19	24.49	24.85	25.29	25.81	26.40	27.06	27.81
7.5	26.71	26.04	25.47	24.98	24.57	24.24	23.98	23.80	23.68	23.62	23.64	23.71	23.85	24.06	24.33	24.67	25.08	25.56	26.12	26.76	27.48
8	26.44	25.80	25.25	24.78	24.41	24.11	23.88	23.71	23.61	23.57	23.59	23.66	23.79	23.98	24.23	24.54	24.92	25.38	25.91	26.53	27.23
8.5	26.24	25.62	25.09	24.65	24.30	24.02	23.82	23.68	23.60	23.57	23.59	23.66	23.78	23.95	24.18	24.47	24.82	25.25	25.76	26.36	27.04
9	26.11	25.50	24.99	24.57	24.24	23.99	23.80	23.68	23.61	23.59	23.61	23.68	23.80	23.96	24.17	24.44	24.77	25.18	25.67	26.25	26.92
9.5	26.05	25.45	24.96	24.55	24.23	23.99	23.82	23.71	23.65	23.63	23.66	23.73	23.83	23.99	24.19	24.45	24.77	25.17	25.64	26.21	26.87
10	26.07	25.47	24.98	24.58	24.27	24.04	23.87	23.76	23.70	23.69	23.72	23.78	23.89	24.04	24.24	24.49	24.81	25.20	25.67	26.23	26.89
10.5	26.16	25.56	25.07	24.67	24.35	24.11	23.95	23.83	23.77	23.76	23.79	23.85	23.96	24.11	24.31	24.57	24.89	25.28	25.75	26.32	26.97
11	26.32	25.72	25.21	24.80	24.48	24.23	24.05	23.93	23.86	23.84	23.87	23.94	24.05	24.20	24.41	24.68	25.01	25.41	25.89	26.47	27.13
11.5	26.55	25.93	25.42	24.99	24.65	24.38	24.18	24.05	23.97	23.94	23.97	24.04	24.15	24.32	24.54	24.82	25.17	25.59	26.09	26.68	27.35
12	26.85	26.22	25.68	25.23	24.87	24.58	24.36	24.20	24.11	24.07	24.09	24.16	24.29	24.47	24.71	25.01	25.38	25.82	26.35	26.95	27.64
12.5	27.21	26.57	26.01	25.53	25.14	24.82	24.58	24.40	24.29	24.24	24.25	24.33	24.46	24.66	24.92	25.25	25.64	26.11	26.66	27.28	27.99
13	27.65	26.98	26.40	25.89	25.47	25.12	24.85	24.65	24.52	24.46	24.47	24.54	24.69	24.90	25.19	25.54	25.97	26.46	27.03	27.68	28.41
13.5	28.14	27.46	26.85	26.32	25.87	25.49	25.19	24.96	24.81	24.74	24.74	24.82	24.98	25.21	25.52	25.90	26.35	26.87	27.47	28.14	28.89
14	28.71	28.00	27.37	26.82	26.34	25.93	25.61	25.36	25.19	25.11	25.10	25.19	25.35	25.60	25.93	26.33	26.81	27.36	27.98	28.67	29.43
14.5	29.34	28.62	27.97	27.39	26.89	26.46	26.11	25.84	25.66	25.56	25.56	25.64	25.82	26.08	26.42	26.85	27.34	27.92	28.56	29.27	30.05
15	30.04	29.31	28.64	28.05	27.52	27.08	26.71	26.42	26.23	26.12	26.11	26.20	26.38	26.65	27.01	27.45	27.96	28.55	29.21	29.94	30.73

Table 9 - Radial basis functions determined by *MATLAB*® for C18(1) phosphates vs time; where phosphate amount 5 means a ratio of K_2HPO_4:KH_2PO_4 of 0.0375:0.0875 g/l, 10 = 0.0750:0.1750 g/l and 15 = 0.1125:0.2625 g/l (Figure 3-6f)

C18(1) / %	**Phosphates**																				
Time / days	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
3	17.56	18.95	20.32	21.67	22.97	24.24	25.45	26.60	27.69	28.71	29.66	30.54	31.35	32.10	32.81	33.48	34.13	34.78	35.42	36.09	36.77
3.2	18.40	18.85	19.91	21.11	22.32	23.51	24.67	25.77	26.82	27.80	28.71	29.55	30.33	31.06	31.74	32.39	33.03	33.66	34.30	34.95	35.63
3.4	19.21	19.34	20.01	20.93	21.96	23.02	24.07	25.09	26.06	26.97	27.83	28.62	29.36	30.05	30.70	31.33	31.94	32.55	33.18	33.82	34.50
3.6	19.98	19.96	20.35	21.02	21.83	22.72	23.63	24.53	25.41	26.24	27.02	27.75	28.43	29.08	29.69	30.28	30.87	31.47	32.07	32.71	33.38
3.8	20.72	20.60	20.80	21.25	21.87	22.58	23.34	24.10	24.86	25.59	26.28	26.94	27.56	28.15	28.72	29.27	29.83	30.40	30.99	31.61	32.28
4	21.44	21.24	21.30	21.58	22.02	22.55	23.15	23.78	24.42	25.04	25.63	26.21	26.75	27.28	27.79	28.30	28.82	29.36	29.93	30.54	31.19
4.2	22.15	21.88	21.83	21.96	22.24	22.62	23.07	23.56	24.07	24.57	25.07	25.55	26.01	26.47	26.92	27.38	27.85	28.36	28.90	29.48	30.13
4.4	22.85	22.52	22.38	22.39	22.53	22.77	23.08	23.44	23.82	24.20	24.59	24.97	25.35	25.73	26.11	26.51	26.93	27.39	27.89	28.46	29.09
4.6	23.56	23.18	22.95	22.86	22.88	22.99	23.17	23.40	23.66	23.93	24.20	24.48	24.77	25.06	25.36	25.69	26.05	26.46	26.93	27.46	28.07
4.8	24.27	23.84	23.54	23.36	23.28	23.28	23.34	23.45	23.59	23.75	23.91	24.09	24.27	24.47	24.69	24.94	25.23	25.58	26.00	26.49	27.08
5	25.00	24.53	24.17	23.91	23.74	23.64	23.59	23.59	23.61	23.66	23.72	23.79	23.87	23.97	24.09	24.25	24.47	24.74	25.10	25.56	26.11
5.2	25.75	25.24	24.83	24.50	24.25	24.06	23.92	23.81	23.73	23.67	23.62	23.58	23.56	23.55	23.57	23.63	23.76	23.96	24.25	24.65	25.17
5.4	26.53	25.98	25.52	25.13	24.81	24.54	24.32	24.12	23.94	23.78	23.62	23.47	23.34	23.22	23.13	23.09	23.11	23.21	23.43	23.77	24.25
5.6	27.33	26.76	26.25	25.82	25.43	25.09	24.79	24.51	24.24	23.98	23.72	23.47	23.22	22.98	22.78	22.61	22.52	22.52	22.64	22.91	23.35
5.8	28.16	27.56	27.03	26.54	26.11	25.71	25.34	24.98	24.63	24.28	23.92	23.56	23.20	22.84	22.51	22.22	21.99	21.87	21.88	22.07	22.46
6	29.02	28.40	27.84	27.32	26.84	26.38	25.95	25.53	25.10	24.66	24.22	23.76	23.28	22.81	22.34	21.91	21.54	21.27	21.15	21.24	21.57
6.2	29.91	29.27	28.68	28.13	27.61	27.12	26.63	26.15	25.65	25.14	24.61	24.05	23.47	22.88	22.29	21.71	21.18	20.75	20.47	20.43	20.69
6.4	30.82	30.17	29.56	28.98	28.43	27.90	27.37	26.83	26.28	25.70	25.10	24.46	23.78	23.08	22.36	21.64	20.94	20.32	19.84	19.63	19.80
6.6	31.76	31.09	30.47	29.87	29.29	28.73	28.16	27.58	26.98	26.35	25.68	24.96	24.21	23.41	22.58	21.73	20.87	20.05	19.33	18.86	18.90
6.8	32.71	32.04	31.40	30.79	30.19	29.60	29.00	28.39	27.74	27.07	26.35	25.58	24.76	23.89	22.97	22.02	21.04	20.05	19.08	18.23	17.97
7	33.69	33.02	32.36	31.74	31.12	30.50	29.88	29.24	28.57	27.86	27.10	26.30	25.44	24.52	23.56	22.55	21.51	20.43	19.32	18.18	17.03

Table 10 - Radial basis functions determined by *MATLAB*® for C18(1) mass nitrates vs iron; where nitrates amount 5 means 0.125 g/l, 10 = 0.250 g/l and 15 = 0.375g/l; where iron amount 5 means 2.49 mg/l, 10 = 4.98 mg/l and 15 = 7.47 mg/l (Figure 3-7a)

C18(1) / mg	Nitrates																				
Iron	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
5	16.59	16.95	17.28	17.57	17.82	18.00	18.10	18.10	17.98	17.71	17.28	16.67	15.86	14.87	13.69	12.33	10.83	9.27	7.77	6.57	6.01
5.5	17.69	18.13	18.56	18.96	19.32	19.62	19.84	19.97	19.96	19.79	19.45	18.91	18.16	17.21	16.07	14.75	13.29	11.76	10.31	9.15	8.58
6	18.78	19.32	19.86	20.38	20.87	21.31	21.67	21.94	22.07	22.03	21.79	21.35	20.68	19.80	18.73	17.48	16.13	14.73	13.41	12.38	11.82
6.5	19.85	20.50	21.15	21.80	22.43	23.03	23.56	24.00	24.29	24.40	24.29	23.95	23.37	22.57	21.58	20.44	19.20	17.96	16.81	15.90	15.36
7	20.88	21.63	22.40	23.19	23.99	24.76	25.48	26.11	26.59	26.87	26.90	26.67	26.17	25.43	24.52	23.47	22.36	21.25	20.24	19.43	18.88
7.5	21.83	22.69	23.59	24.52	25.48	26.44	27.38	28.23	28.93	29.40	29.58	29.44	28.99	28.30	27.42	26.44	25.41	24.41	23.50	22.74	22.18
8	22.68	23.64	24.66	25.73	26.86	28.02	29.18	30.28	31.22	31.91	32.24	32.17	31.74	31.04	30.15	29.19	28.21	27.28	26.42	25.69	25.10
8.5	23.41	24.46	25.58	26.78	28.06	29.41	30.80	32.16	33.38	34.30	34.78	34.76	34.29	33.51	32.57	31.58	30.61	29.70	28.88	28.15	27.53
9	23.98	25.09	26.30	27.61	29.02	30.54	32.13	33.74	35.23	36.40	37.03	37.01	36.44	35.54	34.51	33.47	32.48	31.57	30.75	30.02	29.37
9.5	24.36	25.53	26.79	28.17	29.67	31.30	33.05	34.84	36.55	37.94	38.69	38.64	37.95	36.93	35.81	34.71	33.70	32.79	31.97	31.23	30.56
10	24.56	25.74	27.02	28.43	29.96	31.64	33.44	35.31	37.11	38.59	39.39	39.33	38.59	37.51	36.35	35.23	34.21	33.29	32.47	31.73	31.04
10.5	24.55	25.72	26.99	28.37	29.88	31.52	33.27	35.07	36.79	38.18	38.94	38.90	38.21	37.19	36.07	34.98	33.97	33.06	32.24	31.50	30.82
11	24.35	25.48	26.70	28.02	29.45	30.98	32.59	34.20	35.70	36.89	37.53	37.51	36.95	36.06	35.04	34.00	33.01	32.11	31.29	30.55	29.89
11.5	23.96	25.03	26.17	27.39	28.70	30.07	31.48	32.85	34.09	35.03	35.52	35.51	35.05	34.29	33.36	32.38	31.41	30.50	29.68	28.95	28.31
12	23.42	24.40	25.45	26.54	27.70	28.88	30.07	31.19	32.16	32.87	33.22	33.17	32.76	32.06	31.20	30.24	29.27	28.34	27.48	26.74	26.14
12.5	22.74	23.63	24.56	25.53	26.52	27.51	28.48	29.36	30.08	30.58	30.79	30.67	30.24	29.57	28.71	27.73	26.72	25.72	24.81	24.05	23.47
13	21.96	22.75	23.56	24.39	25.22	26.03	26.78	27.45	27.96	28.27	28.33	28.12	27.65	26.94	26.04	25.01	23.91	22.81	21.80	20.98	20.42
13.5	21.10	21.78	22.48	23.17	23.85	24.49	25.06	25.53	25.86	26.01	25.93	25.62	25.07	24.29	23.32	22.20	20.98	19.75	18.61	17.69	17.13
14	20.18	20.77	21.35	21.92	22.45	22.94	23.35	23.66	23.83	23.83	23.63	23.22	22.58	21.73	20.68	19.46	18.12	16.73	15.43	14.39	13.82
14.5	19.24	19.73	20.21	20.66	21.07	21.42	21.70	21.86	21.90	21.77	21.47	20.96	20.25	19.33	18.21	16.91	15.48	13.97	12.53	11.37	10.77
15	18.29	18.70	19.08	19.42	19.72	19.96	20.11	20.16	20.08	19.86	19.47	18.89	18.12	17.16	16.00	14.67	13.20	11.65	10.16	8.96	8.38

Table 11 - Radial basis functions determined by *MATLAB*® for C18(1) mass nitrates vs phosphates; where nitrates amount 5 means 0.125 g/l, 10 = 0.250 g/l and 15 = 0.375g/l; where phosphate amount 5 means a ratio of K_2HPO_4:KH_2PO_4 of 0.0375:0.0875 g/l, 10 = 0.0750:0.1750 g/l and 15 = 0.1125:0.2625 g/l (Figure 3-7b)

C18(1) / mg	Nitrates																				
Phosphates	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
5	24.03	24.83	25.65	26.48	27.32	28.15	28.99	29.82	30.63	31.43	32.23	33.03	33.86	34.73	35.65	36.64	37.69	38.76	39.73	40.37	40.36
5.5	24.31	25.16	26.04	26.93	27.82	28.72	29.60	30.45	31.28	32.06	32.81	33.54	34.25	34.98	35.75	36.57	37.44	38.33	39.11	39.58	39.46
6	24.57	25.49	26.42	27.38	28.35	29.31	30.24	31.14	31.98	32.75	33.44	34.06	34.64	35.18	35.73	36.31	36.91	37.50	37.98	38.19	37.92
6.5	24.81	25.79	26.79	27.83	28.87	29.91	30.93	31.89	32.76	33.51	34.14	34.65	35.04	35.37	35.65	35.92	36.20	36.45	36.59	36.51	36.10
7	25.01	26.05	27.13	28.25	29.38	30.52	31.63	32.67	33.60	34.36	34.94	35.31	35.51	35.58	35.57	35.52	35.45	35.35	35.17	34.84	34.29
7.5	25.16	26.25	27.41	28.61	29.85	31.10	32.33	33.49	34.50	35.30	35.83	36.08	36.07	35.87	35.55	35.17	34.77	34.34	33.88	33.35	32.69
8	25.23	26.38	27.60	28.89	30.23	31.61	32.98	34.29	35.43	36.30	36.82	36.95	36.73	36.25	35.62	34.93	34.23	33.54	32.85	32.15	31.41
8.5	25.22	26.41	27.68	29.04	30.49	31.99	33.52	35.00	36.31	37.31	37.86	37.88	37.44	36.69	35.79	34.83	33.89	32.99	32.14	31.33	30.55
9	25.11	26.32	27.63	29.04	30.56	32.17	33.84	35.50	37.02	38.20	38.81	38.75	38.11	37.13	36.01	34.86	33.77	32.76	31.82	30.96	30.17
9.5	24.89	26.10	27.41	28.84	30.39	32.07	33.84	35.65	37.36	38.73	39.44	39.33	38.56	37.44	36.21	35.01	33.88	32.85	31.92	31.08	30.32
10	24.56	25.74	27.02	28.43	29.96	31.64	33.44	35.31	37.11	38.59	39.39	39.33	38.59	37.51	36.35	35.23	34.21	33.29	32.47	31.73	31.04
10.5	24.13	25.25	26.47	27.80	29.26	30.87	32.60	34.41	36.16	37.63	38.49	38.59	38.08	37.26	36.38	35.53	34.77	34.09	33.48	32.92	32.36
11	23.61	24.65	25.76	26.98	28.32	29.79	31.37	33.03	34.63	36.00	36.92	37.26	37.15	36.78	36.33	35.92	35.56	35.26	34.97	34.65	34.27
11.5	23.03	23.96	24.95	26.01	27.18	28.46	29.86	31.32	32.76	34.04	35.03	35.67	36.00	36.17	36.29	36.43	36.61	36.79	36.91	36.93	36.76
12	22.42	23.23	24.05	24.94	25.91	26.98	28.17	29.44	30.74	31.98	33.09	34.03	34.83	35.57	36.32	37.09	37.90	38.66	39.30	39.71	39.80
12.5	21.80	22.47	23.13	23.81	24.57	25.44	26.42	27.53	28.72	29.96	31.22	32.47	33.73	35.04	36.43	37.90	39.39	40.83	42.06	42.93	43.31
13	21.19	21.72	22.20	22.68	23.23	23.88	24.69	25.66	26.79	28.06	29.48	31.03	32.73	34.59	36.62	38.79	41.02	43.18	45.08	46.48	47.18
13.5	20.60	20.99	21.29	21.56	21.90	22.37	23.02	23.88	24.97	26.30	27.88	29.72	31.82	34.19	36.82	39.66	42.63	45.55	48.18	50.18	51.21
14	20.00	20.26	20.37	20.45	20.60	20.91	21.44	22.22	23.30	24.70	26.43	28.52	30.96	33.77	36.92	40.37	44.01	47.67	51.04	53.67	55.06
14.5	19.31	19.46	19.41	19.33	19.33	19.52	19.96	20.70	21.78	23.24	25.10	27.39	30.12	33.27	36.83	40.74	44.92	49.18	53.19	56.41	58.13
15	18.41	18.47	18.33	18.15	18.07	18.19	18.58	19.30	20.40	21.92	23.88	26.32	29.23	32.62	36.44	40.65	45.15	49.76	54.13	57.68	59.62

Table 12 - Radial basis functions determined by *MATLAB*® for C18(1) mass nitrates vs time; where nitrates amount 5 means 0.125 g/l, 10 = 0.250 g/l and 15 = 0.375g/l (Figure 3-7c)

C18(1) / mg	Nitrates																				
Time / days	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
3	5.76	5.78	5.81	5.87	5.97	6.12	6.34	6.63	7.00	7.47	8.02	8.67	9.40	10.19	11.04	11.91	12.79	13.66	14.49	15.27	15.98
3.2	7.81	7.94	8.09	8.26	8.48	8.75	9.07	9.46	9.91	10.42	11.01	11.65	12.34	13.08	13.85	14.63	15.41	16.17	16.89	17.56	18.16
3.4	9.93	10.19	10.47	10.78	11.14	11.55	12.00	12.50	13.05	13.63	14.24	14.87	15.51	16.17	16.83	17.49	18.14	18.77	19.36	19.89	20.36
3.6	12.10	12.49	12.92	13.39	13.90	14.47	15.08	15.73	16.39	17.04	17.68	18.29	18.86	19.40	19.92	20.43	20.92	21.38	21.81	22.20	22.52
3.8	14.26	14.79	15.38	16.02	16.71	17.46	18.25	19.06	19.86	20.61	21.27	21.84	22.31	22.71	23.06	23.37	23.67	23.95	24.20	24.42	24.58
4	16.37	17.05	17.80	18.61	19.49	20.44	21.43	22.44	23.40	24.25	24.95	25.46	25.80	26.01	26.14	26.23	26.31	26.38	26.44	26.48	26.47
4.2	18.38	19.21	20.11	21.09	22.16	23.32	24.53	25.75	26.90	27.88	28.61	29.04	29.21	29.19	29.06	28.90	28.74	28.59	28.45	28.30	28.13
4.4	20.25	21.21	22.25	23.39	24.64	25.99	27.42	28.86	30.23	31.37	32.13	32.46	32.41	32.11	31.69	31.26	30.84	30.47	30.13	29.80	29.48
4.6	21.93	22.99	24.15	25.42	26.81	28.33	29.96	31.62	33.21	34.51	35.32	35.51	35.20	34.60	33.88	33.17	32.52	31.93	31.40	30.92	30.45
4.8	23.37	24.51	25.75	27.11	28.61	30.25	32.01	33.83	35.59	37.03	37.87	37.92	37.35	36.46	35.47	34.53	33.66	32.89	32.20	31.57	30.98
5	24.56	25.74	27.02	28.43	29.96	31.64	33.44	35.31	37.11	38.59	39.39	39.33	38.59	37.51	36.35	35.23	34.21	33.29	32.47	31.73	31.04
5.2	25.47	26.65	27.94	29.33	30.84	32.48	34.21	35.98	37.66	39.00	39.69	39.57	38.79	37.67	36.45	35.24	34.12	33.10	32.18	31.36	30.61
5.4	26.10	27.26	28.50	29.83	31.26	32.77	34.35	35.91	37.34	38.43	38.95	38.80	38.08	37.02	35.81	34.59	33.42	32.33	31.35	30.47	29.69
5.6	26.46	27.57	28.73	29.96	31.26	32.60	33.96	35.26	36.39	37.20	37.55	37.36	36.70	35.72	34.56	33.35	32.16	31.03	30.00	29.09	28.29
5.8	26.58	27.60	28.66	29.77	30.90	32.04	33.16	34.19	35.03	35.59	35.77	35.52	34.89	33.96	32.85	31.65	30.43	29.27	28.20	27.26	26.47
6	26.49	27.41	28.35	29.31	30.26	31.20	32.08	32.85	33.44	33.77	33.79	33.46	32.82	31.91	30.81	29.60	28.36	27.15	26.03	25.06	24.29
6.2	26.21	27.02	27.84	28.64	29.42	30.16	30.82	31.36	31.72	31.86	31.73	31.32	30.63	29.70	28.59	27.35	26.06	24.78	23.59	22.58	21.82
6.4	25.79	26.49	27.17	27.83	28.44	28.99	29.45	29.79	29.96	29.93	29.67	29.17	28.42	27.46	26.31	25.02	23.66	22.29	21.01	19.94	19.20
6.6	25.26	25.84	26.40	26.91	27.37	27.76	28.04	28.21	28.22	28.05	27.67	27.08	26.27	25.27	24.08	22.74	21.30	19.84	18.46	17.31	16.56
6.8	24.65	25.12	25.56	25.94	26.26	26.50	26.64	26.66	26.53	26.24	25.77	25.11	24.25	23.21	21.99	20.62	19.13	17.60	16.13	14.91	14.17
7	23.98	24.36	24.68	24.95	25.15	25.26	25.28	25.18	24.94	24.56	24.01	23.30	22.41	21.34	20.12	18.74	17.26	15.72	14.24	13.01	12.30

Table 13 - Radial basis functions determined by *MATLAB*® for C18(1) mass iron vs time; where iron amount 5 means 2.49 mg/l, 10 = 4.98 mg/l and 15 = 7.47 mg/l (Figure 3-7d)

C18(1) / mg	Iron																				
Time / days	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
3	4.61	5.19	5.75	6.29	6.78	7.22	7.57	7.84	8.01	8.07	8.02	7.87	7.60	7.24	6.79	6.26	5.68	5.06	4.43	3.79	3.17
3.2	6.14	6.89	7.64	8.36	9.05	9.67	10.20	10.61	10.89	11.03	11.01	10.83	10.50	10.02	9.43	8.74	7.98	7.18	6.36	5.55	4.77
3.4	7.70	8.63	9.58	10.52	11.42	12.26	12.99	13.58	14.00	14.23	14.24	14.04	13.62	13.02	12.26	11.38	10.42	9.41	8.39	7.38	6.43
3.6	9.25	10.38	11.55	12.72	13.87	14.94	15.90	16.70	17.29	17.62	17.68	17.45	16.94	16.19	15.24	14.15	12.96	11.72	10.48	9.27	8.13
3.8	10.76	12.10	13.49	14.91	16.31	17.65	18.87	19.91	20.69	21.16	21.27	21.01	20.40	19.48	18.31	16.98	15.54	14.06	12.60	11.18	9.85
4	12.21	13.74	15.36	17.03	18.70	20.32	21.83	23.13	24.14	24.77	24.95	24.65	23.91	22.80	21.40	19.81	18.12	16.40	14.70	13.08	11.57
4.2	13.55	15.27	17.10	19.01	20.95	22.86	24.66	26.25	27.52	28.35	28.61	28.28	27.39	26.05	24.40	22.56	20.62	18.66	16.75	14.95	13.27
4.4	14.75	16.64	18.66	20.79	22.97	25.16	27.25	29.15	30.72	31.76	32.13	31.75	30.70	29.13	27.22	25.13	22.96	20.80	18.71	16.74	14.92
4.6	15.78	17.81	19.99	22.30	24.69	27.11	29.47	31.67	33.53	34.83	35.32	34.89	33.65	31.85	29.72	27.42	25.07	22.75	20.53	18.44	16.51
4.8	16.63	18.75	21.04	23.48	26.02	28.61	31.17	33.60	35.72	37.25	37.87	37.40	36.01	34.04	31.76	29.33	26.88	24.48	22.18	20.02	18.03
5	17.28	19.45	21.79	24.29	26.90	29.58	32.24	34.78	37.03	38.69	39.39	38.94	37.53	35.52	33.22	30.79	28.33	25.93	23.63	21.47	19.47
5.2	17.72	19.89	22.23	24.72	27.32	29.98	32.62	35.13	37.34	38.97	39.69	39.33	38.07	36.22	34.06	31.75	29.41	27.11	24.88	22.78	20.82
5.4	17.95	20.07	22.35	24.78	27.29	29.84	32.36	34.72	36.76	38.25	38.95	38.75	37.75	36.19	34.30	32.24	30.12	28.00	25.94	23.96	22.08
5.6	17.99	20.02	22.20	24.49	26.86	29.24	31.56	33.70	35.53	36.87	37.55	37.50	36.80	35.60	34.06	32.33	30.51	28.65	26.81	25.01	23.27
5.8	17.85	19.75	21.79	23.92	26.09	28.27	30.36	32.27	33.89	35.09	35.77	35.87	35.45	34.61	33.45	32.09	30.62	29.08	27.52	25.94	24.38
6	17.55	19.31	21.18	23.11	25.08	27.03	28.88	30.58	32.01	33.11	33.79	34.04	33.87	33.36	32.58	31.61	30.52	29.34	28.09	26.78	25.41
6.2	17.13	18.73	20.41	22.15	23.89	25.61	27.25	28.74	30.02	31.03	31.73	32.11	32.17	31.97	31.54	30.96	30.25	29.44	28.54	27.52	26.37
6.4	16.61	18.05	19.54	21.07	22.61	24.11	25.54	26.85	28.00	28.95	29.67	30.16	30.43	30.49	30.40	30.17	29.84	29.41	28.86	28.16	27.24
6.6	16.03	17.30	18.61	19.95	21.28	22.59	23.83	24.99	26.03	26.93	27.67	28.26	28.69	29.00	29.19	29.28	29.30	29.23	29.04	28.64	27.96
6.8	15.42	16.53	17.67	18.83	19.98	21.11	22.19	23.21	24.16	25.01	25.77	26.44	27.02	27.51	27.94	28.31	28.62	28.86	28.98	28.88	28.42
7	14.79	15.76	16.75	17.74	18.73	19.71	20.65	21.56	22.43	23.24	24.01	24.74	25.42	26.06	26.67	27.25	27.79	28.26	28.62	28.74	28.47

Table 14 - Radial basis functions determined by *MATLAB*® for C18(1) mass iron vs phosphates; where iron amount 5 means 2.49 mg/l, 10 = 4.98 mg/l and 15 = 7.47 mg/l; where phosphate amount 5 means a ratio of K_2HPO_4:KH_2PO_4 of 0.0375:0.0875 g/l, 10 = 0.0750:0.1750 g/l and 15 = 0.1125:0.2625 g/l (Figure 3-7e)

C18(1) / mg	Iron																				
Phosphates	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
5	31.95	32.77	33.21	33.42	33.51	33.52	33.47	33.33	33.09	32.73	32.23	31.58	30.79	29.86	28.81	27.67	26.46	25.20	23.93	22.67	21.43
5.5	31.14	32.09	32.69	33.07	33.34	33.52	33.63	33.64	33.52	33.25	32.81	32.19	31.39	30.42	29.31	28.09	26.79	25.44	24.08	22.73	21.41
6	29.89	31.00	31.82	32.46	32.97	33.39	33.71	33.91	33.95	33.81	33.44	32.85	32.04	31.02	29.84	28.53	27.13	25.68	24.22	22.77	21.37
6.5	28.39	29.66	30.73	31.64	32.44	33.14	33.73	34.16	34.41	34.41	34.14	33.59	32.76	31.69	30.41	28.99	27.48	25.91	24.34	22.79	21.30
7	26.77	28.21	29.51	30.71	31.82	32.83	33.71	34.43	34.91	35.09	34.94	34.42	33.57	32.42	31.03	29.48	27.83	26.13	24.44	22.79	21.20
7.5	25.12	26.71	28.25	29.73	31.14	32.48	33.69	34.71	35.46	35.86	35.83	35.35	34.45	33.19	31.67	29.96	28.16	26.32	24.51	22.75	21.07
8	23.48	25.22	26.97	28.71	30.43	32.09	33.65	35.01	36.07	36.71	36.82	36.37	35.39	33.99	32.29	30.42	28.45	26.47	24.52	22.65	20.89
8.5	21.87	23.74	25.68	27.67	29.67	31.66	33.55	35.27	36.67	37.59	37.86	37.41	36.32	34.74	32.85	30.79	28.66	26.53	24.46	22.49	20.64
9	20.29	22.29	24.40	26.59	28.85	31.13	33.35	35.41	37.16	38.38	38.81	38.36	37.12	35.35	33.26	31.02	28.74	26.48	24.31	22.25	20.33
9.5	18.77	20.86	23.10	25.47	27.94	30.45	32.94	35.30	37.36	38.85	39.44	38.97	37.60	35.65	33.41	31.04	28.64	26.29	24.04	21.91	19.94
10	17.28	19.45	21.79	24.29	26.90	29.58	32.24	34.78	37.03	38.69	39.39	38.94	37.53	35.52	33.22	30.79	28.33	25.93	23.63	21.47	19.47
10.5	15.83	18.05	20.46	23.04	25.74	28.49	31.23	33.82	36.09	37.75	38.49	38.12	36.81	34.89	32.64	30.24	27.80	25.39	23.08	20.90	18.89
11	14.41	16.65	19.10	21.72	24.45	27.22	29.93	32.47	34.64	36.20	36.92	36.66	35.55	33.83	31.73	29.43	27.04	24.67	22.38	20.22	18.22
11.5	13.02	15.26	17.71	20.34	23.07	25.81	28.45	30.87	32.90	34.34	35.03	34.88	33.97	32.47	30.56	28.39	26.11	23.80	21.55	19.42	17.45
12	11.64	13.87	16.32	18.93	21.63	24.32	26.88	29.19	31.08	32.42	33.09	33.02	32.28	30.97	29.24	27.22	25.03	22.80	20.60	18.51	16.59
12.5	10.28	12.48	14.91	17.52	20.19	22.83	25.31	27.51	29.30	30.57	31.22	31.22	30.60	29.44	27.86	25.96	23.87	21.71	19.55	17.51	15.64
13	8.94	11.10	13.53	16.12	18.78	21.37	23.79	25.91	27.62	28.83	29.48	29.52	29.00	27.95	26.48	24.69	22.68	20.56	18.44	16.43	14.61
13.5	7.64	9.77	12.19	14.79	17.44	20.00	22.36	24.42	26.07	27.25	27.88	27.96	27.50	26.54	25.16	23.44	21.49	19.41	17.31	15.33	13.55
14	6.43	8.53	10.96	13.57	16.21	18.74	21.05	23.06	24.66	25.80	26.43	26.53	26.12	25.22	23.91	22.26	20.36	18.30	16.22	14.24	12.52
14.5	5.42	7.49	9.92	12.52	15.13	17.62	19.88	21.82	23.38	24.48	25.10	25.22	24.85	24.01	22.77	21.18	19.33	17.31	15.25	13.29	11.61
15	4.77	6.78	9.16	11.71	14.25	16.66	18.84	20.71	22.21	23.28	23.88	24.01	23.68	22.90	21.72	20.21	18.44	16.50	14.50	12.60	10.99

Table 15 -Radial basis functions determined by *MATLAB*® for C18(1) mass phosphates vs time; where phosphate amount 5 means a ratio of K_2HPO_4:KH_2PO_4 of 0.0375:0.0875 g/l, 10 = 0.0750:0.1750 g/l and 15 = 0.1125:0.2625 g/l (Figure 3-7f)

C18(1) / mg	**Phosphates**																				
Time / days	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
3	-1.84	-1.38	-0.33	1.03	2.45	3.81	5.04	6.10	6.96	7.60	8.02	8.22	8.19	7.94	7.49	6.87	6.10	5.22	4.34	3.59	3.18
3.2	1.18	1.67	2.71	4.05	5.47	6.83	8.07	9.13	9.98	10.62	11.01	11.16	11.07	10.76	10.24	9.55	8.71	7.77	6.84	6.03	5.54
3.4	4.88	5.37	6.33	7.56	8.90	10.21	11.41	12.45	13.28	13.89	14.24	14.34	14.19	13.80	13.21	12.46	11.57	10.61	9.66	8.85	8.29
3.6	8.93	9.42	10.29	11.40	12.62	13.85	15.00	16.00	16.81	17.38	17.68	17.71	17.48	17.01	16.34	15.51	14.58	13.60	12.65	11.82	11.19
3.8	13.05	13.56	14.36	15.37	16.49	17.64	18.73	19.71	20.49	21.02	21.27	21.22	20.89	20.30	19.52	18.61	17.62	16.60	15.63	14.75	14.04
4	17.07	17.61	18.37	19.30	20.35	21.44	22.50	23.46	24.24	24.75	24.95	24.80	24.33	23.59	22.67	21.64	20.55	19.47	18.44	17.50	16.69
4.2	20.87	21.44	22.18	23.06	24.05	25.11	26.16	27.14	27.95	28.47	28.61	28.34	27.69	26.76	25.65	24.46	23.25	22.08	20.97	19.96	19.04
4.4	24.34	24.94	25.66	26.52	27.47	28.51	29.58	30.61	31.47	32.03	32.13	31.72	30.85	29.67	28.34	26.96	25.61	24.32	23.12	22.01	21.00
4.6	27.43	28.04	28.75	29.56	30.49	31.51	32.60	33.68	34.63	35.25	35.32	34.73	33.60	32.15	30.57	28.99	27.49	26.08	24.79	23.59	22.50
4.8	30.07	30.67	31.36	32.13	33.00	33.98	35.05	36.15	37.16	37.83	37.87	37.10	35.71	33.99	32.19	30.44	28.80	27.29	25.91	24.64	23.47
5	32.23	32.81	33.44	34.14	34.94	35.83	36.82	37.86	38.81	39.44	39.39	38.49	36.92	35.03	33.09	31.22	29.48	27.88	26.43	25.10	23.88
5.2	33.91	34.44	34.99	35.60	36.27	37.02	37.85	38.70	39.46	39.89	39.69	38.70	37.10	35.18	33.20	31.28	29.49	27.84	26.34	24.98	23.73
5.4	35.10	35.56	36.02	36.50	37.01	37.58	38.18	38.76	39.21	39.35	38.95	37.92	36.37	34.52	32.58	30.67	28.86	27.18	25.65	24.26	23.01
5.6	35.85	36.22	36.56	36.89	37.23	37.57	37.91	38.19	38.31	38.14	37.55	36.47	34.97	33.21	31.34	29.46	27.65	25.96	24.41	23.01	21.76
5.8	36.20	36.45	36.66	36.84	36.99	37.11	37.18	37.16	36.98	36.54	35.77	34.62	33.15	31.45	29.63	27.77	25.96	24.25	22.67	21.27	20.05
6	36.19	36.33	36.41	36.42	36.39	36.29	36.12	35.83	35.39	34.73	33.79	32.57	31.09	29.41	27.60	25.75	23.91	22.16	20.55	19.12	17.93
6.2	35.89	35.92	35.86	35.72	35.51	35.22	34.83	34.32	33.66	32.81	31.73	30.43	28.91	27.21	25.40	23.52	21.63	19.81	18.14	16.68	15.51
6.4	35.37	35.28	35.09	34.81	34.44	33.98	33.41	32.72	31.88	30.87	29.67	28.29	26.72	24.99	23.14	21.21	19.26	17.35	15.58	14.06	12.91
6.6	34.66	34.46	34.16	33.76	33.25	32.64	31.92	31.08	30.10	28.96	27.67	26.21	24.59	22.83	20.94	18.96	16.93	14.92	13.04	11.44	10.29
6.8	33.82	33.53	33.12	32.61	31.99	31.26	30.42	29.46	28.37	27.14	25.77	24.26	22.60	20.81	18.89	16.88	14.80	12.72	10.75	9.07	7.92
7	32.91	32.52	32.02	31.42	30.71	29.88	28.95	27.90	26.73	25.43	24.01	22.46	20.79	18.99	17.07	15.06	12.98	10.89	8.91	7.22	6.10

Table 16 - Error graphs for C18(1) FAME content derived from algal cells: (1) percentage and (2) mass produced (Nitrates – $NaNO_3$, phosphates – K_2HPO_4:KH_2PO_4, iron – $FeSO_4.7H_2O$, time – days after inoculation) with key to error (red high error, blue low error) (Figure 3-6, Figure 3-7)

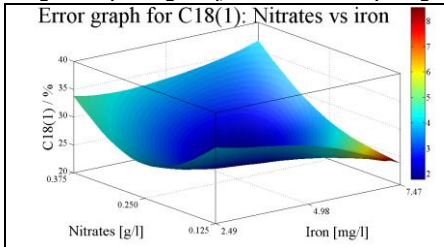
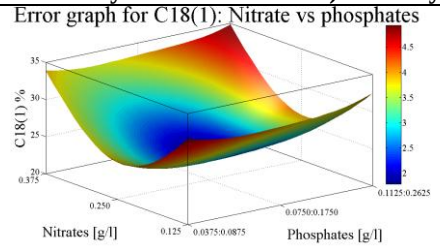
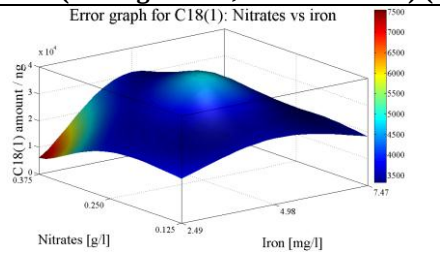
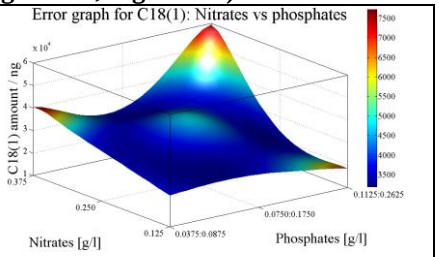
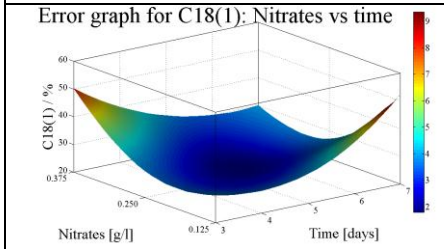
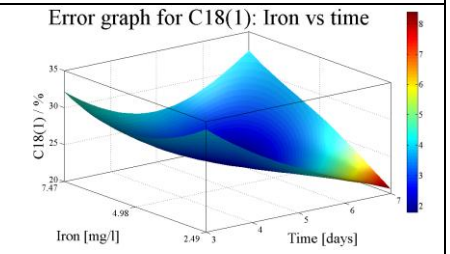
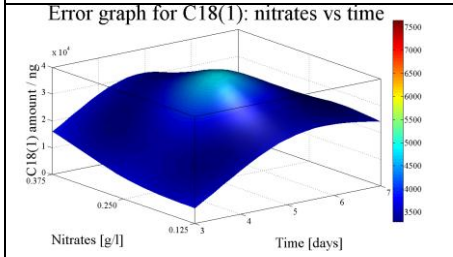
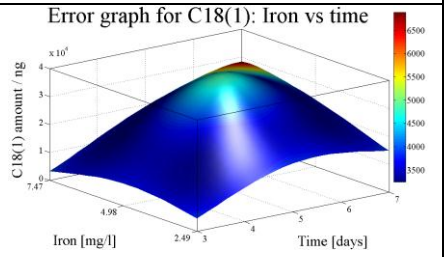
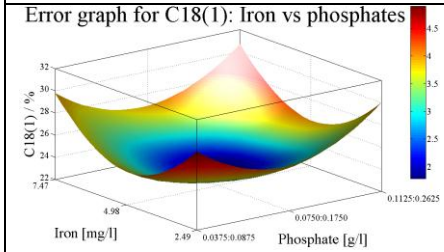
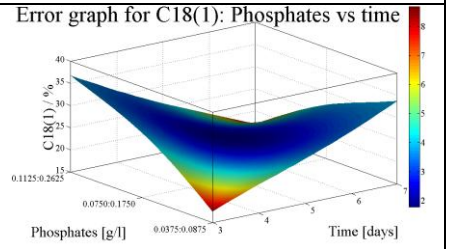
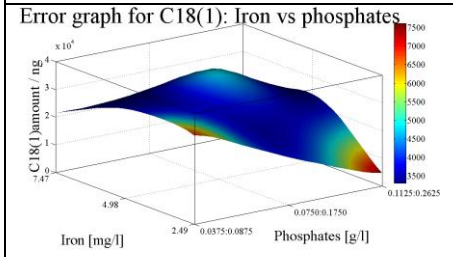
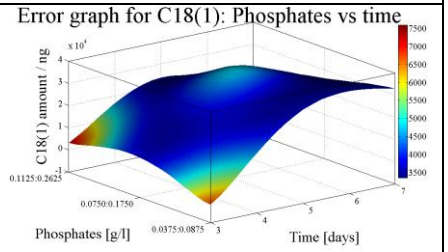
 **1a**	 **1b**	 **2a**	 **2b**
 **1c**	 **1d**	 **2c**	 **2d**
 **1e**	 **1f**	 **2e**	 **2f**

Table 17 - Radial basis functions determined by *MATLAB*® for C16(4) nitrates vs iron; where nitrates amount 5 means 0.125 g/l, 10 = 0.250 g/l and 15 = 0.375g/l; where iron amount 5 means 2.49 mg/l, 10 = 4.98 mg/l and 15 = 7.47 mg/l (Figure 3-8a)

C16(4) / %	**Nitrates**																				
Iron	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
5	3.51	3.48	3.45	3.44	3.44	3.45	3.48	3.52	3.58	3.65	3.74	3.85	3.98	4.13	4.30	4.49	4.69	4.92	5.17	5.44	5.72
5.5	3.38	3.34	3.31	3.29	3.29	3.30	3.32	3.35	3.41	3.48	3.56	3.67	3.80	3.94	4.10	4.29	4.49	4.71	4.94	5.18	5.35
6	3.25	3.21	3.17	3.15	3.14	3.14	3.16	3.19	3.24	3.30	3.38	3.48	3.60	3.74	3.90	4.07	4.26	4.46	4.66	4.85	5.00
6.5	3.13	3.08	3.04	3.01	3.00	2.99	3.00	3.03	3.07	3.13	3.20	3.30	3.41	3.53	3.68	3.84	4.01	4.19	4.37	4.53	4.66
7	3.01	2.96	2.92	2.88	2.86	2.85	2.86	2.88	2.91	2.96	3.03	3.11	3.21	3.33	3.46	3.60	3.76	3.92	4.08	4.22	4.34
7.5	2.91	2.85	2.80	2.76	2.74	2.72	2.72	2.73	2.76	2.80	2.86	2.93	3.02	3.13	3.24	3.37	3.51	3.65	3.79	3.93	4.05
8	2.81	2.75	2.70	2.65	2.62	2.60	2.59	2.59	2.61	2.65	2.70	2.76	2.84	2.93	3.04	3.15	3.28	3.41	3.53	3.66	3.77
8.5	2.73	2.66	2.60	2.55	2.51	2.49	2.47	2.47	2.48	2.51	2.55	2.60	2.67	2.75	2.85	2.95	3.06	3.18	3.29	3.41	3.52
9	2.65	2.58	2.52	2.47	2.42	2.39	2.37	2.36	2.37	2.38	2.42	2.46	2.52	2.59	2.67	2.76	2.86	2.97	3.08	3.19	3.30
9.5	2.59	2.51	2.45	2.39	2.34	2.31	2.28	2.27	2.27	2.28	2.30	2.34	2.39	2.45	2.52	2.60	2.69	2.79	2.89	3.00	3.11
10	2.54	2.46	2.39	2.33	2.28	2.24	2.21	2.19	2.18	2.19	2.20	2.23	2.28	2.33	2.39	2.46	2.55	2.63	2.73	2.83	2.94
10.5	2.50	2.42	2.35	2.29	2.23	2.19	2.15	2.13	2.12	2.12	2.13	2.15	2.18	2.23	2.29	2.35	2.42	2.51	2.60	2.70	2.80
11	2.47	2.39	2.32	2.25	2.20	2.15	2.11	2.09	2.07	2.07	2.07	2.09	2.12	2.15	2.20	2.26	2.33	2.41	2.49	2.59	2.69
11.5	2.45	2.37	2.30	2.24	2.18	2.13	2.09	2.06	2.04	2.03	2.04	2.05	2.07	2.10	2.15	2.20	2.26	2.33	2.42	2.51	2.60
12	2.45	2.37	2.30	2.23	2.17	2.13	2.08	2.05	2.03	2.02	2.02	2.03	2.04	2.07	2.11	2.16	2.22	2.28	2.36	2.45	2.54
12.5	2.46	2.38	2.31	2.24	2.18	2.13	2.09	2.06	2.04	2.02	2.02	2.02	2.04	2.06	2.10	2.14	2.19	2.26	2.33	2.41	2.51
13	2.48	2.40	2.33	2.26	2.21	2.16	2.12	2.08	2.06	2.04	2.04	2.04	2.05	2.07	2.10	2.14	2.19	2.25	2.32	2.40	2.49
13.5	2.51	2.43	2.36	2.30	2.24	2.19	2.15	2.12	2.09	2.08	2.07	2.07	2.08	2.09	2.12	2.16	2.21	2.26	2.33	2.41	2.49
14	2.54	2.47	2.40	2.34	2.28	2.23	2.19	2.16	2.14	2.12	2.11	2.11	2.12	2.13	2.16	2.19	2.24	2.29	2.35	2.43	2.51
14.5	2.59	2.52	2.45	2.39	2.34	2.29	2.25	2.22	2.19	2.18	2.17	2.16	2.17	2.18	2.21	2.24	2.28	2.33	2.39	2.46	2.54
15	2.64	2.57	2.51	2.45	2.40	2.35	2.31	2.28	2.26	2.24	2.23	2.23	2.23	2.24	2.27	2.30	2.33	2.38	2.44	2.51	2.58

Table 18 - Radial basis functions determined by *MATLAB*® for C16(4) nitrates vs phosphates; where nitrates amount 5 means 0.125 g/l, 10 = 0.250 g/l and 15 = 0.375g/l; where phosphate amount 5 means a ratio of K_2HPO_4:KH_2PO_4 of 0.0375:0.0875 g/l, 10 = 0.0750:0.1750 g/l and 15 = 0.1125:0.2625 g/l (Figure 3-8b)

C16(4) / %	**Nitrates**																				
Phosphates	5	5.5	6	6.5	7	7.5	8	8.5	9	9.5	10	10.5	11	11.5	12	12.5	13	13.5	14	14.5	15
5	3.32	3.24	3.17	3.10	3.04	2.99	2.95	2.92	2.90	2.90	2.91	2.94	2.98	3.03	3.10	3.18	3.26	3.35	3.45	3.56	3.66
5.5	3.24	3.16	3.09	3.02	2.97	2.92	2.88	2.85	2.83	2.83	2.84	2.87	2.91	2.96	3.03	3.11	3.19	3.28	3.38	3.48	3.59
6	3.16	3.09	3.02	2.95	2.89	2.85	2.81	2.78	2.77	2.77	2.78	2.81	2.85	2.90	2.97	3.04	3.13	3.22	3.31	3.42	3.52
6.5	3.09	3.01	2.94	2.88	2.83	2.78	2.74	2.72	2.71	2.71	2.72	2.75	2.79	2.84	2.91	2.98	3.06	3.15	3.25	3.35	3.46
7	3.01	2.94	2.87	2.81	2.76	2.72	2.68	2.66	2.65	2.65	2.66	2.69	2.73	2.79	2.85	2.92	3.00	3.09	3.19	3.29	3.39
7.5	2.94	2.87	2.80	2.74	2.69	2.65	2.62	2.60	2.59	2.59	2.61	2.64	2.68	2.73	2.79	2.86	2.94	3.03	3.12	3.22	3.32
8	2.86	2.79	2.73	2.67	2.62	2.58	2.55	2.53	2.53	2.53	2.55	2.57	2.61	2.67	2.73	2.80	2.88	2.96	3.05	3.15	3.25
8.5	2.79	2.71	2.65	2.59	2.55	2.51	2.48	2.46	2.45	2.46	2.48	2.50	2.54	2.60	2.66	2.73	2.81	2.89	2.98	3.08	3.18
9	2.71	2.63	2.57	2.51	2.46	2.43	2.40	2.38	2.38	2.38	2.40	2.43	2.47	2.52	2.58	2.65	2.73	2.81	2.91	3.00	3.11
9.5	2.62	2.55	2.48	2.43	2.38	2.34	2.31	2.29	2.28	2.29	2.31	2.34	2.38	2.43	2.49	2.56	2.64	2.73	2.82	2.92	3.03
10	2.54	2.46	2.39	2.33	2.28	2.24	2.21	2.19	2.18	2.19	2.20	2.23	2.28	2.33	2.39	2.46	2.55	2.63	2.73	2.83	2.94
10.5	2.45	2.37	2.29	2.23	2.18	2.13	2.10	2.08	2.07	2.07	2.09	2.12	2.16	2.22	2.28	2.36	2.44	2.53	2.63	2.74	2.85
11	2.35	2.27	2.19	2.13	2.07	2.02	1.98	1.96	1.95	1.95	1.97	2.00	2.04	2.10	2.16	2.24	2.33	2.43	2.53	2.64	2.75
11.5	2.26	2.17	2.09	2.02	1.95	1.90	1.86	1.83	1.82	1.82	1.83	1.86	1.91	1.97	2.04	2.12	2.21	2.31	2.42	2.54	2.66
12	2.17	2.07	1.99	1.91	1.84	1.78	1.73	1.70	1.68	1.68	1.70	1.73	1.77	1.83	1.91	2.00	2.09	2.20	2.31	2.43	2.56
12.5	2.08	1.98	1.88	1.80	1.72	1.66	1.61	1.57	1.55	1.55	1.56	1.59	1.64	1.70	1.78	1.87	1.97	2.09	2.21	2.33	2.46
13	1.99	1.89	1.79	1.70	1.61	1.54	1.49	1.45	1.42	1.41	1.42	1.46	1.50	1.57	1.65	1.75	1.86	1.98	2.10	2.23	2.37
13.5	1.91	1.80	1.70	1.60	1.51	1.44	1.37	1.33	1.30	1.29	1.30	1.33	1.38	1.45	1.54	1.64	1.75	1.87	2.00	2.14	2.28
14	1.85	1.73	1.62	1.51	1.42	1.34	1.27	1.22	1.19	1.18	1.19	1.22	1.27	1.34	1.43	1.54	1.65	1.78	1.92	2.06	2.20
14.5	1.79	1.66	1.55	1.44	1.34	1.26	1.19	1.13	1.10	1.08	1.09	1.12	1.18	1.25	1.34	1.45	1.57	1.70	1.84	1.98	2.13
15	1.74	1.61	1.49	1.38	1.28	1.19	1.12	1.06	1.03	1.01	1.02	1.05	1.10	1.18	1.27	1.38	1.50	1.63	1.78	1.92	2.08

Table 19 – Radial basis functions determined by *MATLAB*® for C16(4) nitrates vs time; where nitrates amount 5 means 0.125 g/l, 10 = 0.250 g/l and 15 = 0.375g/l (Figure 3-8c)

C16(4) / %	Nitrates																				
Time / days	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
3	2.64	2.57	2.51	2.47	2.43	2.40	2.39	2.39	2.40	2.42	2.45	2.50	2.55	2.62	2.70	2.79	2.88	2.98	3.09	3.20	3.31
3.2	2.58	2.51	2.45	2.40	2.36	2.34	2.32	2.32	2.33	2.35	2.38	2.43	2.49	2.56	2.64	2.73	2.82	2.92	3.03	3.14	3.26
3.4	2.53	2.46	2.40	2.35	2.31	2.28	2.26	2.25	2.26	2.28	2.32	2.37	2.43	2.50	2.58	2.67	2.77	2.87	2.98	3.10	3.21
3.6	2.49	2.42	2.35	2.30	2.26	2.23	2.21	2.20	2.21	2.23	2.27	2.31	2.37	2.45	2.53	2.62	2.72	2.83	2.94	3.06	3.17
3.8	2.46	2.39	2.32	2.27	2.22	2.19	2.17	2.16	2.17	2.19	2.22	2.27	2.33	2.40	2.49	2.58	2.68	2.79	2.90	3.02	3.14
4	2.44	2.37	2.30	2.24	2.20	2.16	2.14	2.13	2.14	2.16	2.19	2.24	2.30	2.37	2.45	2.55	2.65	2.76	2.87	2.99	3.11
4.2	2.44	2.36	2.29	2.23	2.19	2.15	2.13	2.12	2.12	2.14	2.17	2.21	2.27	2.34	2.43	2.52	2.62	2.73	2.84	2.96	3.07
4.4	2.44	2.37	2.30	2.24	2.19	2.15	2.13	2.11	2.11	2.13	2.16	2.20	2.26	2.33	2.41	2.50	2.60	2.70	2.81	2.93	3.04
4.6	2.46	2.38	2.32	2.26	2.21	2.17	2.14	2.12	2.12	2.14	2.16	2.20	2.25	2.32	2.39	2.48	2.58	2.68	2.79	2.90	3.01
4.8	2.49	2.42	2.35	2.29	2.24	2.20	2.17	2.15	2.15	2.15	2.18	2.21	2.26	2.32	2.39	2.47	2.56	2.66	2.76	2.87	2.98
5	2.54	2.46	2.39	2.33	2.28	2.24	2.21	2.19	2.18	2.19	2.20	2.23	2.28	2.33	2.39	2.46	2.55	2.63	2.73	2.83	2.94
5.2	2.59	2.52	2.45	2.39	2.34	2.30	2.27	2.24	2.23	2.23	2.25	2.27	2.30	2.35	2.40	2.46	2.53	2.61	2.70	2.80	2.90
5.4	2.66	2.58	2.52	2.46	2.41	2.37	2.34	2.31	2.30	2.29	2.30	2.31	2.34	2.37	2.41	2.46	2.52	2.59	2.67	2.76	2.85
5.6	2.74	2.66	2.60	2.54	2.49	2.45	2.42	2.39	2.38	2.37	2.36	2.37	2.38	2.41	2.43	2.47	2.52	2.57	2.63	2.71	2.80
5.8	2.82	2.75	2.69	2.64	2.59	2.55	2.51	2.49	2.47	2.45	2.44	2.44	2.44	2.45	2.46	2.48	2.51	2.55	2.60	2.66	2.74
6	2.92	2.85	2.79	2.74	2.69	2.65	2.62	2.59	2.56	2.54	2.53	2.51	2.50	2.50	2.49	2.50	2.51	2.53	2.56	2.61	2.68
6.2	3.02	2.96	2.90	2.85	2.81	2.77	2.73	2.70	2.67	2.65	2.62	2.60	2.58	2.56	2.54	2.52	2.51	2.50	2.52	2.55	2.61
6.4	3.13	3.07	3.02	2.97	2.92	2.89	2.85	2.82	2.79	2.75	2.72	2.69	2.66	2.62	2.59	2.55	2.52	2.49	2.48	2.48	2.53
6.6	3.24	3.19	3.14	3.09	3.05	3.01	2.97	2.94	2.90	2.87	2.83	2.79	2.75	2.70	2.65	2.60	2.54	2.49	2.44	2.42	2.45
6.8	3.36	3.31	3.26	3.21	3.17	3.13	3.10	3.06	3.03	2.99	2.94	2.90	2.85	2.79	2.73	2.66	2.59	2.51	2.43	2.37	2.36
7	3.48	3.43	3.38	3.34	3.30	3.26	3.23	3.19	3.15	3.11	3.06	3.01	2.95	2.89	2.82	2.74	2.66	2.57	2.47	2.37	2.26

Table 20 – Radial basis functions determined by *MATLAB*® for C16(4) iron vs time; where iron amount 5 means 2.49 mg/l, 10 = 4.98 mg/l and 15 = 7.47 mg/l (Figure 3-8d)

C16(4) / %	Iron																				
Time / days	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
3	3.21	3.10	2.99	2.89	2.80	2.71	2.64	2.58	2.53	2.48	2.45	2.43	2.42	2.41	2.42	2.43	2.45	2.48	2.52	2.57	2.62
3.2	3.18	3.06	2.94	2.84	2.75	2.66	2.58	2.52	2.46	2.42	2.38	2.36	2.34	2.34	2.34	2.35	2.38	2.41	2.45	2.50	2.55
3.4	3.16	3.03	2.91	2.81	2.71	2.62	2.54	2.47	2.41	2.36	2.32	2.29	2.27	2.27	2.27	2.29	2.31	2.34	2.38	2.43	2.48
3.6	3.15	3.02	2.90	2.78	2.68	2.58	2.50	2.42	2.36	2.31	2.27	2.24	2.22	2.21	2.21	2.22	2.25	2.28	2.32	2.37	2.43
3.8	3.17	3.03	2.90	2.78	2.67	2.57	2.48	2.40	2.33	2.27	2.22	2.19	2.17	2.16	2.16	2.17	2.19	2.22	2.26	2.32	2.37
4	3.20	3.05	2.92	2.79	2.67	2.57	2.47	2.38	2.30	2.24	2.19	2.15	2.12	2.11	2.11	2.12	2.14	2.18	2.22	2.27	2.33
4.2	3.25	3.10	2.96	2.83	2.70	2.58	2.48	2.38	2.30	2.23	2.17	2.12	2.09	2.08	2.07	2.08	2.10	2.14	2.18	2.23	2.29
4.4	3.33	3.18	3.02	2.88	2.74	2.62	2.50	2.40	2.30	2.22	2.16	2.11	2.07	2.05	2.04	2.05	2.07	2.10	2.15	2.20	2.26
4.6	3.44	3.27	3.12	2.96	2.81	2.67	2.55	2.43	2.32	2.23	2.16	2.10	2.06	2.04	2.03	2.03	2.05	2.08	2.13	2.18	2.24
4.8	3.57	3.40	3.23	3.07	2.91	2.75	2.61	2.48	2.36	2.26	2.18	2.11	2.06	2.03	2.02	2.02	2.04	2.07	2.11	2.17	2.23
5	3.74	3.56	3.38	3.20	3.03	2.86	2.70	2.55	2.42	2.30	2.20	2.13	2.07	2.04	2.02	2.02	2.04	2.07	2.11	2.17	2.23
5.2	3.95	3.76	3.57	3.37	3.17	2.98	2.80	2.64	2.49	2.36	2.25	2.16	2.09	2.05	2.03	2.03	2.04	2.07	2.12	2.18	2.24
5.4	4.18	3.99	3.78	3.56	3.35	3.13	2.93	2.74	2.57	2.42	2.30	2.20	2.13	2.08	2.05	2.05	2.06	2.09	2.14	2.20	2.26
5.6	4.46	4.25	4.03	3.79	3.55	3.31	3.08	2.87	2.67	2.51	2.36	2.25	2.17	2.11	2.08	2.08	2.09	2.12	2.17	2.23	2.30
5.8	4.77	4.55	4.30	4.04	3.77	3.50	3.24	3.00	2.79	2.60	2.44	2.32	2.22	2.16	2.13	2.12	2.13	2.17	2.22	2.28	2.34
6	5.11	4.88	4.61	4.32	4.01	3.71	3.42	3.15	2.91	2.70	2.53	2.39	2.29	2.22	2.18	2.17	2.19	2.22	2.27	2.34	2.40
6.2	5.50	5.25	4.95	4.62	4.27	3.93	3.60	3.31	3.04	2.81	2.62	2.47	2.36	2.28	2.24	2.23	2.25	2.29	2.35	2.41	2.48
6.4	5.91	5.65	5.30	4.92	4.53	4.15	3.79	3.47	3.18	2.93	2.72	2.56	2.44	2.36	2.32	2.31	2.33	2.37	2.43	2.50	2.57
6.6	6.36	6.07	5.66	5.22	4.78	4.36	3.97	3.62	3.31	3.05	2.83	2.66	2.53	2.45	2.40	2.39	2.41	2.46	2.53	2.60	2.68
6.8	6.85	6.48	5.98	5.48	5.00	4.55	4.14	3.77	3.45	3.17	2.94	2.76	2.63	2.54	2.49	2.48	2.50	2.55	2.63	2.72	2.80
7	7.37	6.77	6.20	5.67	5.17	4.70	4.28	3.91	3.58	3.29	3.06	2.88	2.74	2.64	2.59	2.58	2.60	2.65	2.73	2.82	2.94

Table 21 – Radial basis functions determined by *MATLAB*® for C16(4) iron vs phosphates; where iron amount 5 means 2.49 mg/l, 10 = 4.98 mg/l and 15 = 7.47 mg/l; where phosphate amount 5 means a ratio of K_2HPO_4:KH_2PO_4 of 0.0375:0.0875 g/l, 10 = 0.0750:0.1750 g/l and 15 = 0.1125:0.2625 g/l (Figure 3-8e)

C16(4) / %	Iron																				
Phosphates	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
5	4.82	4.62	4.40	4.19	3.98	3.77	3.57	3.38	3.20	3.05	2.91	2.80	2.71	2.65	2.61	2.59	2.59	2.60	2.63	2.67	2.73
5.5	4.78	4.57	4.36	4.14	3.93	3.71	3.51	3.32	3.14	2.98	2.84	2.73	2.64	2.57	2.53	2.51	2.51	2.52	2.56	2.60	2.65
6	4.74	4.53	4.32	4.10	3.88	3.66	3.46	3.26	3.08	2.92	2.78	2.66	2.57	2.51	2.46	2.44	2.44	2.46	2.49	2.53	2.59
6.5	4.69	4.48	4.27	4.05	3.83	3.61	3.40	3.21	3.02	2.86	2.72	2.60	2.51	2.45	2.40	2.38	2.38	2.40	2.43	2.47	2.53
7	4.63	4.43	4.21	3.99	3.77	3.56	3.35	3.15	2.97	2.80	2.66	2.55	2.46	2.39	2.35	2.33	2.33	2.34	2.37	2.42	2.47
7.5	4.56	4.35	4.14	3.92	3.71	3.49	3.28	3.09	2.91	2.75	2.61	2.49	2.40	2.34	2.30	2.28	2.28	2.29	2.33	2.37	2.43
8	4.46	4.26	4.05	3.84	3.62	3.41	3.21	3.01	2.84	2.68	2.55	2.43	2.35	2.29	2.25	2.23	2.23	2.25	2.28	2.33	2.38
8.5	4.33	4.14	3.93	3.73	3.52	3.31	3.11	2.93	2.76	2.61	2.48	2.37	2.29	2.23	2.19	2.18	2.18	2.20	2.24	2.28	2.34
9	4.18	3.98	3.79	3.59	3.38	3.19	3.00	2.82	2.66	2.52	2.40	2.30	2.22	2.17	2.14	2.13	2.13	2.16	2.19	2.24	2.30
9.5	3.98	3.79	3.60	3.41	3.22	3.04	2.86	2.70	2.55	2.42	2.31	2.22	2.15	2.11	2.08	2.07	2.09	2.11	2.15	2.20	2.27
10	3.74	3.56	3.38	3.20	3.03	2.86	2.70	2.55	2.42	2.30	2.20	2.13	2.07	2.04	2.02	2.02	2.04	2.07	2.11	2.17	2.23
10.5	3.47	3.29	3.12	2.96	2.80	2.65	2.51	2.38	2.27	2.17	2.09	2.03	1.98	1.96	1.95	1.96	1.98	2.02	2.07	2.13	2.19
11	3.14	2.98	2.82	2.68	2.54	2.42	2.30	2.20	2.10	2.03	1.97	1.92	1.89	1.88	1.88	1.90	1.93	1.97	2.02	2.09	2.16
11.5	2.78	2.63	2.49	2.36	2.25	2.16	2.07	1.99	1.93	1.87	1.83	1.81	1.79	1.79	1.81	1.84	1.87	1.92	1.98	2.05	2.12
12	2.38	2.24	2.12	2.02	1.94	1.88	1.82	1.78	1.74	1.71	1.70	1.69	1.69	1.71	1.73	1.77	1.82	1.88	1.94	2.01	2.09
12.5	1.95	1.81	1.72	1.66	1.62	1.59	1.57	1.56	1.55	1.55	1.56	1.57	1.59	1.62	1.66	1.71	1.77	1.83	1.90	1.98	2.06
13	1.48	1.36	1.30	1.28	1.29	1.30	1.32	1.35	1.37	1.40	1.42	1.46	1.50	1.54	1.59	1.65	1.71	1.79	1.86	1.94	2.03
13.5	0.99	0.89	0.88	0.91	0.96	1.03	1.09	1.14	1.20	1.25	1.30	1.35	1.41	1.47	1.53	1.60	1.67	1.75	1.83	1.92	2.01
14	0.47	0.41	0.46	0.56	0.67	0.78	0.87	0.96	1.04	1.12	1.19	1.26	1.33	1.40	1.47	1.55	1.63	1.71	1.80	1.89	1.99
14.5	-0.06	-0.05	0.11	0.27	0.43	0.57	0.70	0.81	0.91	1.00	1.09	1.18	1.26	1.34	1.43	1.51	1.60	1.69	1.78	1.88	1.97
15	-0.61	-0.36	-0.13	0.08	0.26	0.42	0.56	0.69	0.81	0.92	1.02	1.11	1.21	1.30	1.39	1.48	1.58	1.67	1.77	1.87	1.97

Table 22 – Radial basis functions determined by *MATLAB*® for C16(4) phosphates vs time; where phosphate amount 5 means a ratio of K_2HPO_4:KH_2PO_4 of 0.0375:0.0875 g/l, 10 = 0.0750:0.1750 g/l and 15 = 0.1125:0.2625 g/l (Figure 3-8f)

C16(4) / %	Phosphates																				
Time / days	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
3	3.59	3.48	3.37	3.26	3.15	3.04	2.92	2.81	2.69	2.57	2.45	2.33	2.21	2.09	1.98	1.87	1.78	1.69	1.61	1.54	1.49
3.2	3.52	3.41	3.30	3.19	3.08	2.97	2.86	2.74	2.63	2.50	2.38	2.26	2.13	2.01	1.89	1.78	1.68	1.58	1.50	1.43	1.38
3.4	3.45	3.34	3.24	3.13	3.02	2.91	2.80	2.69	2.57	2.44	2.32	2.19	2.06	1.94	1.81	1.70	1.59	1.49	1.40	1.33	1.27
3.6	3.38	3.28	3.18	3.07	2.97	2.86	2.75	2.63	2.52	2.39	2.27	2.14	2.01	1.87	1.75	1.62	1.51	1.40	1.31	1.24	1.18
3.8	3.32	3.22	3.12	3.02	2.91	2.81	2.70	2.59	2.47	2.35	2.22	2.09	1.96	1.82	1.69	1.56	1.44	1.33	1.24	1.16	1.09
4	3.25	3.16	3.06	2.96	2.87	2.77	2.66	2.55	2.44	2.32	2.19	2.06	1.92	1.78	1.65	1.52	1.39	1.28	1.18	1.09	1.03
4.2	3.19	3.09	3.00	2.91	2.82	2.73	2.63	2.52	2.41	2.29	2.17	2.04	1.90	1.76	1.62	1.49	1.36	1.24	1.13	1.05	0.98
4.4	3.12	3.03	2.95	2.86	2.78	2.69	2.60	2.50	2.39	2.28	2.16	2.03	1.89	1.75	1.61	1.47	1.34	1.22	1.11	1.02	0.95
4.6	3.05	2.97	2.89	2.82	2.74	2.66	2.58	2.49	2.39	2.28	2.16	2.03	1.90	1.76	1.62	1.48	1.35	1.22	1.11	1.02	0.95
4.8	2.98	2.91	2.84	2.77	2.70	2.63	2.56	2.48	2.39	2.29	2.18	2.06	1.93	1.79	1.65	1.51	1.37	1.25	1.14	1.04	0.97
5	2.91	2.84	2.78	2.72	2.66	2.61	2.55	2.48	2.40	2.31	2.20	2.09	1.97	1.83	1.70	1.56	1.42	1.30	1.19	1.09	1.02
5.2	2.83	2.77	2.72	2.67	2.63	2.59	2.54	2.48	2.42	2.34	2.25	2.14	2.02	1.90	1.76	1.63	1.50	1.37	1.26	1.17	1.09
5.4	2.75	2.69	2.65	2.62	2.59	2.57	2.54	2.50	2.44	2.38	2.30	2.20	2.10	1.98	1.85	1.72	1.59	1.47	1.36	1.27	1.19
5.6	2.66	2.61	2.58	2.57	2.56	2.55	2.54	2.52	2.48	2.43	2.36	2.28	2.18	2.07	1.95	1.83	1.71	1.59	1.48	1.39	1.31
5.8	2.56	2.52	2.51	2.51	2.52	2.53	2.54	2.54	2.53	2.49	2.44	2.37	2.28	2.18	2.07	1.96	1.84	1.72	1.62	1.53	1.45
6	2.45	2.42	2.43	2.45	2.49	2.52	2.56	2.58	2.58	2.56	2.53	2.47	2.40	2.31	2.20	2.09	1.98	1.87	1.77	1.68	1.61
6.2	2.33	2.32	2.34	2.39	2.46	2.52	2.58	2.62	2.64	2.64	2.62	2.58	2.52	2.44	2.34	2.24	2.14	2.04	1.94	1.85	1.78
6.4	2.20	2.20	2.25	2.34	2.43	2.53	2.61	2.67	2.71	2.73	2.72	2.69	2.64	2.58	2.49	2.40	2.30	2.20	2.11	2.03	1.95
6.6	2.05	2.08	2.17	2.30	2.43	2.55	2.65	2.73	2.79	2.82	2.83	2.81	2.78	2.72	2.64	2.56	2.47	2.38	2.29	2.21	2.14
6.8	1.90	1.97	2.12	2.29	2.45	2.59	2.71	2.81	2.88	2.92	2.94	2.94	2.91	2.86	2.80	2.72	2.64	2.55	2.47	2.39	2.32
7	1.72	1.94	2.15	2.34	2.51	2.66	2.79	2.90	2.98	3.03	3.06	3.06	3.04	3.00	2.95	2.88	2.80	2.72	2.65	2.57	2.51

Table 23 - Radial basis functions determined by *MATLAB*® for C16(4) mass nitrates vs iron; where nitrates amount 5 means 0.125 g/l, 10 = 0.250 g/l and 15 = 0.375g/l; where iron amount 5 means 2.49 mg/l, 10 = 4.98 mg/l and 15 = 7.47 mg/l (Figure 3-9a)

C16(4) / mg	Nitrates																				
Iron	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
5	4.44	4.48	4.45	4.42	4.39	4.35	4.32	4.27	4.21	4.12	4.01	3.86	3.69	3.48	3.25	2.99	2.71	2.41	2.10	1.79	1.54
5.5	4.28	4.37	4.38	4.38	4.37	4.37	4.36	4.33	4.29	4.22	4.11	3.98	3.81	3.60	3.37	3.10	2.82	2.52	2.21	1.91	1.68
6	4.05	4.17	4.24	4.29	4.33	4.36	4.38	4.38	4.37	4.32	4.23	4.11	3.94	3.74	3.50	3.24	2.96	2.66	2.37	2.09	1.87
6.5	3.81	3.97	4.08	4.18	4.26	4.34	4.40	4.44	4.45	4.43	4.36	4.25	4.09	3.89	3.65	3.39	3.11	2.82	2.54	2.28	2.06
7	3.59	3.76	3.92	4.06	4.19	4.31	4.41	4.50	4.55	4.56	4.51	4.41	4.26	4.05	3.81	3.55	3.27	2.99	2.72	2.46	2.24
7.5	3.38	3.58	3.76	3.94	4.11	4.28	4.43	4.57	4.66	4.70	4.68	4.59	4.44	4.23	3.98	3.71	3.43	3.15	2.88	2.63	2.40
8	3.20	3.41	3.62	3.83	4.05	4.26	4.46	4.64	4.79	4.87	4.88	4.80	4.63	4.41	4.15	3.87	3.58	3.29	3.02	2.77	2.54
8.5	3.04	3.26	3.50	3.74	3.98	4.24	4.49	4.73	4.93	5.07	5.10	5.02	4.84	4.59	4.30	4.00	3.70	3.41	3.14	2.89	2.65
9	2.91	3.14	3.39	3.65	3.93	4.22	4.51	4.80	5.07	5.28	5.36	5.26	5.03	4.74	4.42	4.11	3.80	3.50	3.23	2.97	2.74
9.5	2.82	3.05	3.30	3.58	3.87	4.18	4.51	4.84	5.18	5.47	5.61	5.47	5.17	4.84	4.50	4.17	3.86	3.56	3.29	3.03	2.80
10	2.75	2.98	3.24	3.51	3.81	4.13	4.47	4.82	5.18	5.54	5.74	5.55	5.20	4.85	4.50	4.18	3.87	3.58	3.31	3.06	2.83
10.5	2.72	2.95	3.19	3.46	3.75	4.05	4.38	4.71	5.05	5.35	5.49	5.36	5.08	4.76	4.43	4.12	3.83	3.55	3.30	3.06	2.84
11	2.72	2.94	3.17	3.42	3.68	3.96	4.25	4.54	4.81	5.03	5.12	5.04	4.84	4.58	4.30	4.02	3.75	3.49	3.25	3.03	2.82
11.5	2.76	2.96	3.17	3.39	3.62	3.86	4.10	4.34	4.54	4.69	4.75	4.70	4.55	4.35	4.11	3.87	3.63	3.40	3.18	2.97	2.77
12	2.83	3.01	3.20	3.38	3.57	3.76	3.95	4.13	4.28	4.38	4.41	4.37	4.26	4.09	3.90	3.70	3.49	3.28	3.08	2.89	2.71
12.5	2.93	3.09	3.24	3.39	3.53	3.67	3.81	3.93	4.03	4.09	4.10	4.06	3.97	3.84	3.68	3.51	3.33	3.14	2.97	2.79	2.63
13	3.06	3.20	3.31	3.41	3.50	3.59	3.67	3.75	3.80	3.83	3.83	3.78	3.70	3.59	3.46	3.31	3.16	3.00	2.84	2.69	2.54
13.5	3.22	3.33	3.40	3.44	3.48	3.52	3.55	3.58	3.60	3.60	3.58	3.53	3.45	3.36	3.24	3.12	2.98	2.85	2.71	2.57	2.44
14	3.40	3.48	3.49	3.47	3.46	3.44	3.43	3.43	3.41	3.39	3.35	3.30	3.23	3.14	3.04	2.93	2.81	2.69	2.57	2.45	2.33
14.5	3.59	3.61	3.55	3.48	3.42	3.36	3.32	3.28	3.24	3.20	3.15	3.09	3.02	2.94	2.85	2.75	2.65	2.54	2.44	2.33	2.22
15	3.69	3.66	3.55	3.44	3.35	3.27	3.20	3.13	3.08	3.02	2.96	2.90	2.83	2.75	2.67	2.58	2.49	2.40	2.30	2.21	2.11

Table 24 - Radial basis functions determined by *MATLAB*® for C16(4) mass nitrates vs phosphates; where nitrates amount 5 means 0.125 g/l, 10 = 0.250 g/l and 15 = 0.375 g/l; where phosphate amount 5 means a ratio of K_2HPO_4:KH_2PO_4 of 0.0375:0.0875 g/l, 10 = 0.0750:0.1750 g/l and 15 = 0.1125:0.2625 g/l (Figure 3-9b)

C16(4) / mg	Nitrates																				
Phosphates	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
5	1.00	1.23	1.53	1.83	2.13	2.42	2.70	2.98	3.24	3.50	3.76	4.01	4.26	4.52	4.78	5.06	5.34	5.64	5.95	6.25	6.41
5.5	1.21	1.43	1.71	2.01	2.32	2.61	2.89	3.17	3.43	3.68	3.93	4.16	4.39	4.62	4.85	5.09	5.34	5.60	5.85	6.08	6.16
6	1.49	1.69	1.95	2.24	2.54	2.83	3.11	3.39	3.64	3.89	4.11	4.32	4.52	4.71	4.90	5.09	5.27	5.46	5.63	5.75	5.76
6.5	1.77	1.97	2.22	2.49	2.78	3.07	3.35	3.62	3.88	4.11	4.31	4.49	4.65	4.79	4.92	5.05	5.16	5.27	5.36	5.39	5.34
7	2.02	2.23	2.47	2.74	3.03	3.32	3.60	3.87	4.12	4.34	4.52	4.67	4.78	4.87	4.93	4.98	5.03	5.06	5.06	5.03	4.94
7.5	2.25	2.47	2.71	2.98	3.27	3.56	3.85	4.13	4.38	4.59	4.75	4.86	4.91	4.93	4.93	4.91	4.87	4.83	4.76	4.68	4.55
8	2.44	2.67	2.92	3.19	3.48	3.78	4.08	4.37	4.63	4.84	4.98	5.05	5.05	4.99	4.91	4.81	4.71	4.59	4.47	4.33	4.18
8.5	2.60	2.83	3.08	3.36	3.66	3.97	4.28	4.59	4.87	5.09	5.22	5.24	5.17	5.04	4.88	4.70	4.53	4.35	4.18	4.00	3.82
9	2.70	2.93	3.19	3.48	3.78	4.10	4.43	4.76	5.08	5.33	5.46	5.43	5.27	5.05	4.81	4.57	4.33	4.11	3.89	3.68	3.48
9.5	2.75	2.99	3.25	3.53	3.83	4.15	4.50	4.85	5.20	5.52	5.68	5.57	5.30	5.00	4.69	4.39	4.11	3.85	3.60	3.37	3.15
10	2.75	2.98	3.24	3.51	3.81	4.13	4.47	4.82	5.18	5.54	5.74	5.55	5.20	4.85	4.50	4.18	3.87	3.58	3.31	3.06	2.83
10.5	2.69	2.92	3.16	3.43	3.71	4.01	4.33	4.66	4.98	5.26	5.39	5.24	4.94	4.59	4.24	3.91	3.59	3.29	3.02	2.76	2.53
11	2.58	2.80	3.03	3.28	3.54	3.81	4.10	4.38	4.63	4.82	4.89	4.78	4.54	4.24	3.92	3.60	3.29	2.99	2.72	2.47	2.24
11.5	2.42	2.62	2.84	3.06	3.30	3.55	3.79	4.02	4.21	4.34	4.36	4.27	4.08	3.83	3.55	3.25	2.96	2.68	2.42	2.18	1.96
12	2.22	2.40	2.59	2.80	3.01	3.23	3.43	3.61	3.76	3.84	3.85	3.77	3.61	3.40	3.15	2.89	2.63	2.37	2.12	1.90	1.69
12.5	1.97	2.13	2.31	2.50	2.69	2.87	3.04	3.19	3.29	3.35	3.35	3.27	3.14	2.97	2.75	2.52	2.29	2.06	1.83	1.62	1.43
13	1.70	1.84	2.00	2.17	2.34	2.50	2.64	2.76	2.84	2.88	2.87	2.80	2.69	2.54	2.36	2.16	1.95	1.75	1.55	1.36	1.19
13.5	1.39	1.52	1.67	1.82	1.97	2.12	2.24	2.33	2.40	2.42	2.41	2.35	2.26	2.13	1.98	1.81	1.63	1.45	1.27	1.11	0.95
14	1.07	1.19	1.33	1.48	1.62	1.74	1.85	1.93	1.98	2.00	1.98	1.93	1.85	1.74	1.62	1.47	1.32	1.16	1.01	0.86	0.73
14.5	0.75	0.86	1.00	1.15	1.28	1.39	1.48	1.55	1.59	1.60	1.59	1.54	1.47	1.38	1.28	1.15	1.03	0.89	0.76	0.64	0.52
15	0.46	0.57	0.71	0.85	0.97	1.06	1.14	1.20	1.23	1.24	1.22	1.19	1.13	1.05	0.96	0.86	0.75	0.64	0.53	0.42	0.32

Table 25 - Radial basis functions determined by *MATLAB*® for C16(4) mass nitrates vs time; where nitrates amount 5 means 0.125 g/l, 10 = 0.250 g/l and 15 = 0.375g/l (Figure 3-9c)

C16(4) / mg	Nitrates																				
Time / days	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
3	0.75	0.78	0.81	0.84	0.87	0.90	0.92	0.95	0.97	0.98	1.00	1.01	1.02	1.02	1.02	1.02	1.02	1.01	0.99	0.98	0.96
3.2	0.99	1.04	1.09	1.14	1.18	1.23	1.27	1.30	1.33	1.36	1.37	1.38	1.38	1.38	1.37	1.35	1.33	1.30	1.27	1.24	1.20
3.4	1.24	1.30	1.37	1.44	1.51	1.57	1.63	1.69	1.73	1.76	1.78	1.79	1.78	1.76	1.73	1.70	1.65	1.61	1.55	1.50	1.45
3.6	1.48	1.57	1.66	1.75	1.85	1.94	2.02	2.10	2.16	2.20	2.23	2.23	2.21	2.17	2.12	2.06	1.99	1.92	1.84	1.77	1.69
3.8	1.72	1.84	1.95	2.07	2.19	2.31	2.42	2.53	2.61	2.67	2.70	2.70	2.66	2.60	2.52	2.43	2.33	2.23	2.13	2.03	1.93
4	1.96	2.09	2.23	2.38	2.53	2.68	2.83	2.97	3.08	3.17	3.20	3.19	3.13	3.04	2.93	2.80	2.67	2.53	2.40	2.28	2.15
4.2	2.17	2.33	2.50	2.68	2.86	3.05	3.24	3.42	3.57	3.68	3.73	3.70	3.61	3.48	3.33	3.16	2.99	2.82	2.66	2.51	2.36
4.4	2.37	2.55	2.74	2.95	3.17	3.39	3.63	3.85	4.06	4.21	4.27	4.23	4.10	3.91	3.71	3.49	3.28	3.08	2.89	2.71	2.54
4.6	2.53	2.73	2.95	3.18	3.43	3.70	3.97	4.25	4.52	4.73	4.82	4.75	4.56	4.31	4.04	3.78	3.53	3.30	3.08	2.87	2.68
4.8	2.66	2.88	3.12	3.38	3.65	3.95	4.26	4.59	4.92	5.21	5.36	5.23	4.95	4.63	4.32	4.02	3.73	3.47	3.22	2.99	2.78
5	2.75	2.98	3.24	3.51	3.81	4.13	4.47	4.82	5.18	5.54	5.74	5.55	5.20	4.85	4.50	4.18	3.87	3.58	3.31	3.06	2.83
5.2	2.79	3.04	3.31	3.60	3.90	4.23	4.58	4.93	5.27	5.58	5.72	5.58	5.28	4.94	4.59	4.26	3.93	3.63	3.34	3.08	2.84
5.4	2.79	3.04	3.32	3.62	3.93	4.26	4.60	4.92	5.22	5.45	5.54	5.45	5.22	4.92	4.59	4.26	3.93	3.61	3.31	3.04	2.79
5.6	2.74	3.00	3.28	3.59	3.91	4.23	4.55	4.85	5.10	5.28	5.34	5.27	5.09	4.83	4.52	4.19	3.86	3.54	3.23	2.94	2.69
5.8	2.65	2.91	3.20	3.51	3.83	4.15	4.46	4.73	4.95	5.10	5.14	5.08	4.92	4.69	4.40	4.08	3.74	3.41	3.09	2.80	2.54
6	2.51	2.77	3.07	3.39	3.71	4.03	4.33	4.59	4.79	4.92	4.95	4.90	4.75	4.53	4.25	3.93	3.58	3.24	2.90	2.60	2.34
6.2	2.34	2.60	2.90	3.23	3.57	3.90	4.19	4.44	4.63	4.74	4.77	4.71	4.57	4.36	4.08	3.75	3.40	3.03	2.68	2.35	2.09
6.4	2.12	2.38	2.71	3.06	3.41	3.75	4.04	4.29	4.47	4.58	4.60	4.54	4.40	4.18	3.90	3.57	3.20	2.81	2.42	2.07	1.80
6.6	1.87	2.15	2.50	2.89	3.26	3.60	3.90	4.14	4.32	4.42	4.44	4.38	4.24	4.02	3.73	3.39	3.00	2.58	2.16	1.77	1.47
6.8	1.61	1.92	2.32	2.73	3.12	3.47	3.77	4.01	4.18	4.28	4.30	4.23	4.09	3.87	3.58	3.23	2.83	2.39	1.92	1.47	1.14
7	1.44	1.78	2.21	2.63	3.02	3.36	3.66	3.89	4.05	4.15	4.16	4.10	3.96	3.74	3.45	3.11	2.71	2.26	1.78	1.29	0.92

Table 26 – Radial basis functions determined by *MATLAB*® for C16(4) mass iron vs time; where iron amount 5 means 2.49 mg/l, 10 = 4.98 mg/l and 15 = 7.47 mg/l (Figure 3-9d)

C16(4) / mg	Iron																				
Time / days	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
3	0.87	0.89	0.92	0.95	0.97	1.00	1.02	1.03	1.03	1.02	1.00	0.96	0.90	0.82	0.73	0.62	0.50	0.36	0.21	0.06	-0.06
3.2	1.12	1.16	1.20	1.24	1.28	1.32	1.36	1.38	1.40	1.39	1.37	1.33	1.27	1.19	1.09	0.97	0.84	0.69	0.53	0.38	0.28
3.4	1.39	1.44	1.49	1.55	1.61	1.67	1.72	1.76	1.79	1.80	1.78	1.74	1.68	1.59	1.48	1.35	1.21	1.05	0.90	0.76	0.66
3.6	1.67	1.73	1.80	1.88	1.96	2.04	2.11	2.17	2.22	2.24	2.23	2.19	2.11	2.01	1.89	1.75	1.60	1.44	1.29	1.15	1.04
3.8	1.97	2.04	2.13	2.22	2.32	2.42	2.52	2.61	2.67	2.70	2.70	2.66	2.57	2.46	2.32	2.16	2.00	1.83	1.67	1.53	1.41
4	2.28	2.37	2.47	2.58	2.70	2.82	2.95	3.06	3.15	3.20	3.20	3.15	3.05	2.92	2.75	2.58	2.40	2.22	2.05	1.89	1.75
4.2	2.60	2.70	2.81	2.94	3.08	3.23	3.38	3.52	3.64	3.71	3.73	3.67	3.54	3.37	3.18	2.98	2.77	2.58	2.39	2.22	2.07
4.4	2.93	3.04	3.17	3.31	3.46	3.63	3.81	3.98	4.14	4.25	4.27	4.19	4.03	3.81	3.58	3.35	3.12	2.90	2.70	2.52	2.36
4.6	3.28	3.39	3.52	3.67	3.83	4.02	4.22	4.42	4.63	4.78	4.82	4.71	4.48	4.21	3.93	3.67	3.42	3.18	2.97	2.78	2.60
4.8	3.64	3.75	3.88	4.02	4.18	4.37	4.58	4.81	5.05	5.27	5.36	5.18	4.87	4.54	4.22	3.92	3.65	3.41	3.19	2.99	2.81
5	4.01	4.11	4.23	4.36	4.51	4.68	4.88	5.10	5.36	5.61	5.74	5.49	5.12	4.75	4.41	4.10	3.83	3.58	3.35	3.15	2.96
5.2	4.39	4.49	4.58	4.69	4.81	4.95	5.11	5.29	5.49	5.67	5.72	5.52	5.18	4.83	4.50	4.20	3.92	3.68	3.46	3.25	3.07
5.4	4.79	4.87	4.94	5.01	5.08	5.17	5.27	5.38	5.49	5.57	5.54	5.37	5.10	4.80	4.50	4.21	3.95	3.72	3.51	3.31	3.13
5.6	5.21	5.26	5.30	5.32	5.34	5.36	5.38	5.41	5.44	5.42	5.34	5.18	4.95	4.69	4.42	4.16	3.93	3.71	3.51	3.32	3.15
5.8	5.65	5.68	5.66	5.63	5.57	5.52	5.47	5.41	5.35	5.27	5.14	4.97	4.76	4.53	4.29	4.06	3.85	3.65	3.46	3.29	3.13
6	6.11	6.10	6.04	5.93	5.80	5.67	5.53	5.40	5.26	5.12	4.95	4.77	4.56	4.35	4.14	3.93	3.74	3.56	3.39	3.23	3.09
6.2	6.60	6.55	6.42	6.24	6.02	5.80	5.58	5.37	5.17	4.97	4.77	4.57	4.37	4.16	3.96	3.78	3.60	3.44	3.29	3.15	3.02
6.4	7.11	7.02	6.81	6.53	6.22	5.92	5.62	5.34	5.08	4.83	4.60	4.38	4.17	3.97	3.79	3.62	3.46	3.31	3.18	3.05	2.93
6.6	7.64	7.48	7.17	6.79	6.39	6.00	5.64	5.30	4.99	4.70	4.44	4.21	3.99	3.79	3.61	3.45	3.31	3.18	3.06	2.95	2.84
6.8	8.15	7.91	7.46	6.97	6.50	6.04	5.63	5.24	4.89	4.58	4.30	4.04	3.82	3.63	3.45	3.30	3.17	3.04	2.94	2.84	2.74
7	8.47	8.13	7.59	7.04	6.52	6.03	5.58	5.17	4.79	4.46	4.16	3.90	3.67	3.47	3.30	3.16	3.03	2.92	2.82	2.73	2.65

Table 27 - Radial basis functions determined by *MATLAB*® for C16(4) mass iron vs phosphates; where iron amount 5 means 2.49 mg/l, 10 = 4.98 mg/l and 15 = 7.47 mg/l; where phosphate amount 5 means a ratio of K_2HPO_4:KH_2PO_4 of 0.0375:0.0875 g/l, 10 = 0.0750:0.1750 g/l and 15 = 0.1125:0.2625 g/l (Figure 3-9e)

C16(4) / mg	Iron																				
Phosphates	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
5	2.79	2.91	3.07	3.23	3.37	3.49	3.59	3.67	3.72	3.75	3.76	3.74	3.71	3.65	3.59	3.51	3.42	3.32	3.22	3.12	3.02
5.5	3.00	3.10	3.25	3.40	3.54	3.67	3.77	3.84	3.90	3.92	3.93	3.90	3.86	3.79	3.71	3.62	3.52	3.41	3.29	3.18	3.07
6	3.26	3.35	3.47	3.61	3.74	3.86	3.96	4.04	4.09	4.12	4.11	4.08	4.02	3.94	3.85	3.73	3.62	3.49	3.36	3.24	3.11
6.5	3.51	3.59	3.70	3.82	3.95	4.06	4.16	4.24	4.30	4.32	4.31	4.27	4.20	4.10	3.98	3.85	3.71	3.57	3.43	3.29	3.15
7	3.73	3.81	3.92	4.03	4.15	4.27	4.37	4.45	4.51	4.54	4.52	4.47	4.38	4.26	4.12	3.96	3.80	3.64	3.48	3.33	3.18
7.5	3.91	4.00	4.10	4.22	4.34	4.46	4.57	4.66	4.73	4.77	4.75	4.68	4.57	4.42	4.25	4.06	3.88	3.70	3.52	3.36	3.19
8	4.05	4.14	4.25	4.37	4.49	4.62	4.75	4.86	4.95	5.00	4.98	4.90	4.76	4.57	4.36	4.15	3.94	3.74	3.55	3.36	3.19
8.5	4.13	4.23	4.34	4.47	4.60	4.74	4.89	5.03	5.15	5.23	5.22	5.12	4.93	4.70	4.46	4.21	3.97	3.75	3.55	3.35	3.17
9	4.16	4.26	4.38	4.50	4.64	4.80	4.97	5.15	5.32	5.45	5.46	5.33	5.09	4.80	4.51	4.23	3.97	3.73	3.52	3.31	3.13
9.5	4.12	4.22	4.34	4.47	4.62	4.79	4.97	5.19	5.41	5.61	5.68	5.49	5.17	4.83	4.50	4.20	3.92	3.68	3.45	3.25	3.06
10	4.01	4.11	4.23	4.36	4.51	4.68	4.88	5.10	5.36	5.61	5.74	5.49	5.12	4.75	4.41	4.10	3.83	3.58	3.35	3.15	2.96
10.5	3.83	3.93	4.05	4.17	4.32	4.49	4.68	4.89	5.11	5.32	5.39	5.21	4.89	4.55	4.23	3.94	3.67	3.43	3.21	3.02	2.84
11	3.58	3.68	3.79	3.91	4.05	4.20	4.37	4.56	4.73	4.86	4.89	4.76	4.53	4.25	3.98	3.71	3.47	3.25	3.04	2.86	2.69
11.5	3.28	3.36	3.46	3.58	3.71	3.85	4.00	4.14	4.27	4.36	4.36	4.27	4.10	3.89	3.66	3.44	3.23	3.03	2.84	2.67	2.51
12	2.91	2.99	3.08	3.19	3.31	3.44	3.57	3.69	3.79	3.85	3.85	3.78	3.66	3.50	3.32	3.13	2.95	2.78	2.62	2.46	2.32
12.5	2.50	2.57	2.66	2.76	2.88	3.00	3.11	3.22	3.30	3.34	3.35	3.30	3.21	3.09	2.95	2.80	2.66	2.51	2.37	2.24	2.12
13	2.05	2.11	2.20	2.30	2.42	2.54	2.65	2.74	2.82	2.86	2.87	2.84	2.78	2.69	2.58	2.47	2.35	2.23	2.12	2.01	1.90
13.5	1.56	1.62	1.72	1.84	1.96	2.08	2.19	2.28	2.35	2.39	2.41	2.40	2.36	2.30	2.22	2.14	2.04	1.95	1.86	1.77	1.68
14	1.06	1.12	1.24	1.38	1.51	1.64	1.75	1.84	1.91	1.96	1.98	1.98	1.96	1.92	1.87	1.81	1.74	1.67	1.60	1.53	1.45
14.5	0.56	0.64	0.79	0.95	1.10	1.23	1.34	1.43	1.51	1.56	1.59	1.60	1.59	1.57	1.54	1.50	1.45	1.40	1.35	1.29	1.23
15	0.14	0.24	0.41	0.58	0.73	0.86	0.97	1.06	1.13	1.19	1.22	1.24	1.25	1.25	1.23	1.20	1.17	1.14	1.10	1.06	1.02

Table 28 - Radial basis functions determined by *MATLAB*® for C16(4) mass phosphates vs time; where phosphate amount 5 means a ratio of K_2HPO_4:KH_2PO_4 of 0.0375:0.0875 g/l, 10 = 0.0750:0.1750 g/l and 15 = 0.1125:0.2625 g/l (Figure 3-9f)

C16(4) / mg	**Phosphates**																				
Time / days	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
3	-0.03	0.10	0.28	0.46	0.62	0.76	0.87	0.95	1.00	1.01	1.00	0.95	0.87	0.77	0.64	0.50	0.34	0.18	0.01	-0.17	-0.33
3.2	0.35	0.46	0.63	0.81	0.98	1.13	1.24	1.33	1.38	1.40	1.37	1.31	1.22	1.10	0.95	0.78	0.60	0.41	0.21	0.02	-0.17
3.4	0.79	0.90	1.05	1.22	1.39	1.53	1.66	1.75	1.80	1.81	1.78	1.71	1.60	1.45	1.27	1.08	0.86	0.64	0.42	0.20	-0.01
3.6	1.25	1.35	1.49	1.66	1.82	1.97	2.10	2.20	2.25	2.27	2.23	2.14	2.00	1.83	1.62	1.39	1.14	0.89	0.64	0.40	0.16
3.8	1.69	1.80	1.95	2.11	2.27	2.43	2.57	2.67	2.74	2.75	2.70	2.59	2.43	2.22	1.98	1.71	1.43	1.14	0.86	0.59	0.33
4	2.11	2.24	2.39	2.55	2.73	2.89	3.04	3.16	3.24	3.25	3.20	3.08	2.88	2.63	2.34	2.03	1.71	1.39	1.08	0.78	0.49
4.2	2.51	2.65	2.81	2.98	3.17	3.35	3.52	3.66	3.75	3.78	3.73	3.58	3.34	3.05	2.71	2.35	1.99	1.63	1.29	0.96	0.66
4.4	2.87	3.03	3.20	3.38	3.58	3.78	3.97	4.14	4.27	4.32	4.27	4.09	3.81	3.45	3.06	2.66	2.26	1.86	1.49	1.14	0.81
4.6	3.21	3.37	3.55	3.74	3.95	4.16	4.38	4.58	4.76	4.86	4.82	4.60	4.25	3.83	3.38	2.93	2.50	2.07	1.67	1.30	0.96
4.8	3.50	3.67	3.85	4.05	4.27	4.49	4.72	4.96	5.18	5.35	5.36	5.08	4.63	4.15	3.65	3.17	2.70	2.26	1.84	1.45	1.10
5	3.76	3.93	4.11	4.31	4.52	4.75	4.98	5.22	5.46	5.68	5.74	5.39	4.89	4.36	3.85	3.35	2.87	2.41	1.98	1.59	1.22
5.2	3.97	4.14	4.32	4.51	4.71	4.93	5.14	5.36	5.57	5.73	5.72	5.42	4.97	4.47	3.96	3.46	2.98	2.53	2.10	1.70	1.34
5.4	4.14	4.30	4.47	4.65	4.84	5.03	5.22	5.39	5.53	5.61	5.54	5.30	4.92	4.47	4.00	3.52	3.06	2.62	2.20	1.80	1.44
5.6	4.28	4.43	4.58	4.75	4.91	5.07	5.22	5.34	5.43	5.44	5.34	5.12	4.80	4.40	3.98	3.54	3.10	2.67	2.27	1.89	1.53
5.8	4.37	4.51	4.65	4.79	4.93	5.06	5.17	5.25	5.29	5.26	5.14	4.94	4.65	4.30	3.92	3.51	3.11	2.71	2.32	1.95	1.61
6	4.44	4.56	4.68	4.80	4.91	5.01	5.09	5.13	5.13	5.08	4.95	4.76	4.50	4.19	3.84	3.47	3.09	2.72	2.36	2.01	1.68
6.2	4.48	4.58	4.69	4.78	4.87	4.94	4.99	5.00	4.98	4.90	4.77	4.59	4.35	4.06	3.75	3.41	3.07	2.72	2.38	2.05	1.74
6.4	4.49	4.58	4.67	4.74	4.81	4.85	4.87	4.87	4.82	4.73	4.60	4.42	4.20	3.94	3.65	3.35	3.03	2.71	2.39	2.08	1.79
6.6	4.49	4.56	4.63	4.69	4.73	4.75	4.76	4.73	4.67	4.58	4.44	4.27	4.06	3.83	3.56	3.28	2.99	2.69	2.39	2.11	1.83
6.8	4.47	4.53	4.58	4.62	4.65	4.65	4.64	4.60	4.53	4.43	4.30	4.13	3.94	3.71	3.47	3.21	2.94	2.67	2.39	2.12	1.86
7	4.44	4.49	4.52	4.55	4.56	4.55	4.52	4.47	4.40	4.29	4.16	4.00	3.82	3.61	3.39	3.15	2.90	2.65	2.39	2.14	1.89

Table 29 - Error graphs for C16(4) content derived from algal cells: (1) percentage and (2) mass produced (Nitrates – $NaNO_3$, phosphates – K_2HPO_4:KH_2PO_4, iron – $FeSO_4.7H_2O$, time – days after inoculation) with key to error (red high error, blue low error) (Figure 3.8, Figure 3-9)

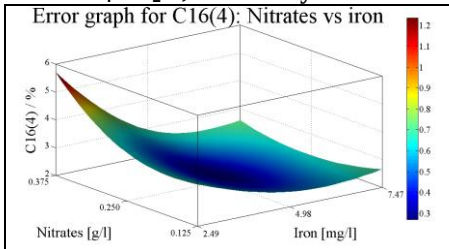
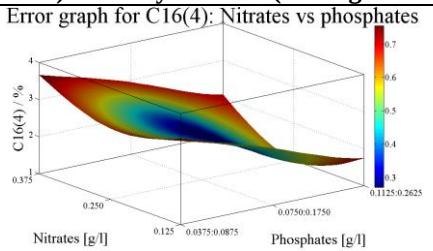
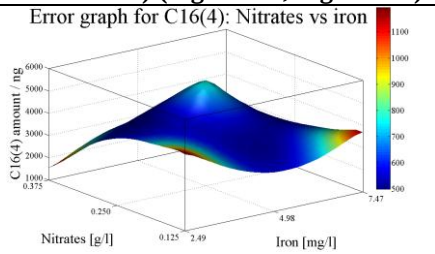
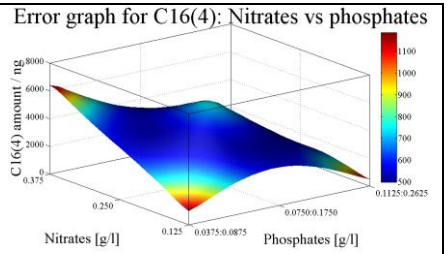
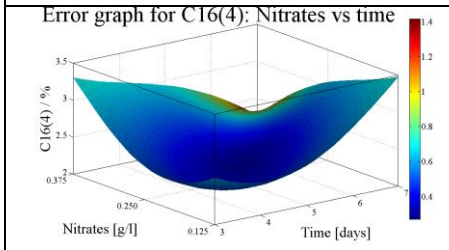
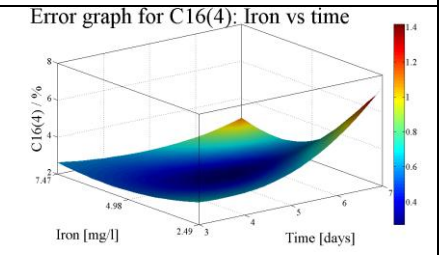
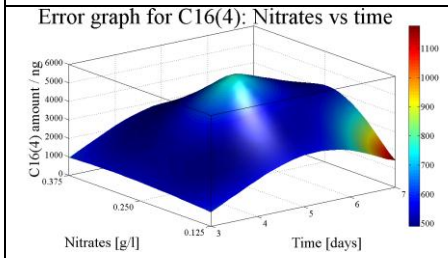
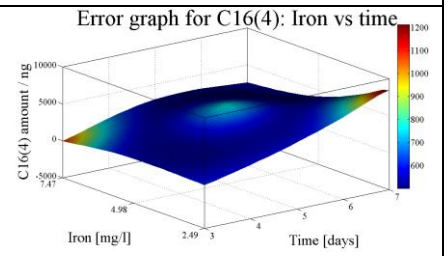
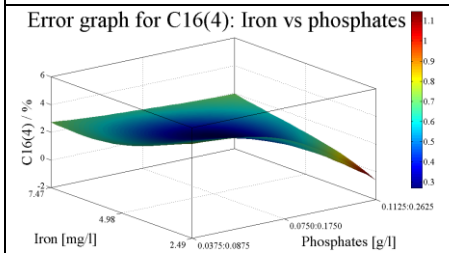
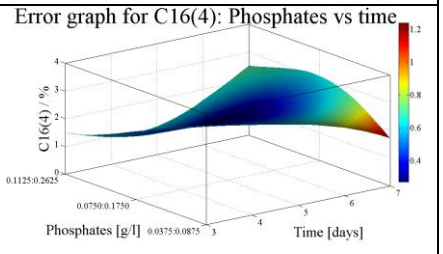
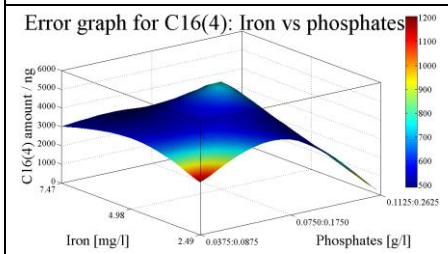
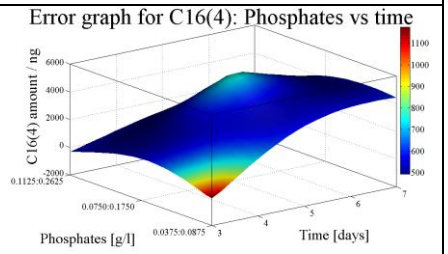
 **1a**	 **1b**	 **2a**	 **2b**
 **1c**	 **1d**	 **2c**	 **2d**
 **1e**	 **1f**	 **2e**	 **2f**

Table 30 - Radial basis functions determined by *MATLAB*® for monounsaturates nitrates vs iron; where nitrates amount 5 means 0.125 g/l, 10 = 0.250 g/l and 15 = 0.375g/l; where iron amount 5 means 2.49 mg/l, 10 = 4.98 mg/l and 15 = 7.47 mg/l (Figure 3.10a)

Monounsaturates / %	**Nitrates**																				
Iron	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
5	24.10	16.08	9.09	2.97	-2.36	-6.94	-10.76	-13.79	-15.98	-17.30	-17.71	-17.21	-15.80	-13.51	-10.39	-6.48	-1.82	3.60	9.80	16.87	24.96
5.5	14.80	8.60	3.16	-1.67	-5.97	-9.73	-12.93	-15.51	-17.40	-18.54	-18.90	-18.45	-17.21	-15.23	-12.55	-9.26	-5.41	-1.03	3.88	9.40	15.67
6	10.22	5.72	1.69	-1.98	-5.35	-8.40	-11.07	-13.27	-14.90	-15.90	-16.21	-15.80	-14.71	-12.97	-10.68	-7.92	-4.78	-1.33	2.43	6.53	11.11
6.5	9.36	6.39	3.64	0.99	-1.57	-4.01	-6.23	-8.12	-9.55	-10.44	-10.71	-10.34	-9.35	-7.82	-5.84	-3.52	-0.99	1.66	4.39	7.22	10.27
7	11.20	9.59	7.96	6.20	4.33	2.40	0.54	-1.10	-2.38	-3.18	-3.43	-3.08	-2.18	-0.80	0.94	2.89	4.92	6.88	8.72	10.44	12.13
7.5	14.76	14.33	13.65	12.64	11.33	9.80	8.23	6.77	5.60	4.86	4.64	4.97	5.81	7.08	8.64	10.31	11.92	13.33	14.42	15.18	15.70
8	19.12	19.65	19.76	19.35	18.48	17.28	15.91	14.58	13.49	12.79	12.58	12.90	13.70	14.90	16.32	17.79	19.09	20.05	20.54	20.52	20.06
8.5	23.43	24.73	25.44	25.50	24.96	23.98	22.76	21.52	20.48	19.80	19.59	19.90	20.69	21.84	23.18	24.50	25.57	26.20	26.23	25.61	24.39
9	27.01	28.85	29.99	30.36	30.06	29.23	28.10	26.91	25.89	25.22	25.01	25.32	26.10	27.23	28.52	29.75	30.67	31.08	30.79	29.74	27.97
9.5	29.32	31.49	32.87	33.43	33.24	32.49	31.40	30.24	29.22	28.55	28.35	28.66	29.44	30.56	31.83	33.01	33.86	34.14	33.67	32.37	30.29
10	30.05	32.30	33.75	34.35	34.19	33.45	32.37	31.20	30.18	29.51	29.31	29.62	30.40	31.52	32.79	33.98	34.81	35.06	34.55	33.19	31.01
10.5	29.07	31.18	32.52	33.03	32.80	32.01	30.89	29.70	28.67	27.99	27.78	28.10	28.88	30.02	31.32	32.54	33.42	33.74	33.32	32.07	30.04
11	26.52	28.26	29.30	29.58	29.19	28.29	27.09	25.85	24.79	24.10	23.89	24.20	25.00	26.17	27.51	28.81	29.81	30.30	30.10	29.15	27.48
11.5	22.73	23.88	24.44	24.36	23.70	22.61	21.29	19.98	18.87	18.16	17.94	18.26	19.08	20.29	21.71	23.12	24.31	25.07	25.23	24.75	23.68
12	18.23	18.57	18.48	17.90	16.87	15.51	14.02	12.59	11.43	10.68	10.45	10.79	11.64	12.91	14.43	16.03	17.47	18.60	19.27	19.44	19.18
12.5	13.75	13.07	12.15	10.93	9.41	7.71	5.98	4.40	3.14	2.34	2.10	2.45	3.35	4.71	6.39	8.21	10.01	11.62	12.93	13.92	14.68
13	10.11	8.21	6.30	4.29	2.18	0.04	-2.00	-3.79	-5.17	-6.04	-6.30	-5.94	-4.97	-3.48	-1.60	0.54	2.77	4.97	7.07	9.06	11.03
13.5	8.25	4.95	1.89	-1.04	-3.87	-6.55	-8.97	-11.02	-12.58	-13.54	-13.83	-13.44	-12.38	-10.72	-8.58	-6.06	-3.29	-0.37	2.64	5.79	9.16
14	9.17	4.30	-0.06	-4.05	-7.72	-11.03	-13.92	-16.30	-18.07	-19.15	-19.48	-19.05	-17.87	-16.01	-13.53	-10.55	-7.15	-3.40	0.68	5.12	10.06
14.5	13.88	7.30	1.49	-3.69	-8.30	-12.35	-15.79	-18.56	-20.60	-21.83	-22.21	-21.73	-20.41	-18.28	-15.41	-11.88	-7.75	-3.05	2.21	8.10	14.76
15	23.40	14.97	7.59	1.10	-4.56	-9.44	-13.52	-16.75	-19.10	-20.51	-20.95	-20.41	-18.91	-16.47	-13.15	-8.99	-4.02	1.73	8.30	15.76	24.26

Table 31 - Radial basis functions determined by *MATLAB*® for monounsaturates nitrates vs phosphates; where nitrates amount 5 means 0.125 g/l, 10 = 0.250 g/l and 15 = 0.375g/l; where phosphates of K_2HPO_4:KH_2PO_4 of 0.0375:0.0875 g/l, 10 = 0.0750:0.1750 g/l and 15 = 0.1125:0.2625 g/l (Figure 3.10b)

Monounsaturates / %	**Nitrates**																				
Phosphates	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
5	54.69	38.64	25.12	13.97	5.05	-1.74	-6.49	-9.27	-10.19	-9.37	-6.98	-3.25	1.56	7.10	12.98	18.76	24.01	28.29	31.25	32.66	32.45
5.5	36.88	23.66	12.67	3.75	-3.21	-8.31	-11.59	-13.12	-12.97	-11.24	-8.09	-3.72	1.59	7.51	13.66	19.58	24.83	28.99	31.68	32.68	31.92
6	24.27	13.67	4.99	-1.94	-7.20	-10.86	-12.95	-13.50	-12.55	-10.20	-6.57	-1.84	3.71	9.77	15.95	21.80	26.88	30.77	33.11	33.67	32.38
6.5	16.17	7.98	1.36	-3.81	-7.63	-10.12	-11.28	-11.12	-9.66	-6.95	-3.10	1.72	7.27	13.22	19.21	24.80	29.56	33.07	34.99	35.09	33.33
7	11.88	5.86	1.08	-2.59	-5.21	-6.78	-7.28	-6.68	-4.96	-2.16	1.66	6.33	11.64	17.28	22.89	28.07	32.38	35.43	36.89	36.55	34.37
7.5	10.71	6.64	3.46	1.06	-0.61	-1.52	-1.61	-0.81	0.92	3.58	7.13	11.44	16.30	21.45	26.53	31.16	34.93	37.46	38.45	37.71	35.21
8	12.00	9.65	7.84	6.46	5.50	5.03	5.12	5.88	7.40	9.70	12.78	16.54	20.79	25.30	29.73	33.72	36.89	38.89	39.43	38.35	35.64
8.5	15.13	14.26	13.58	13.01	12.54	12.28	12.34	12.86	13.96	15.73	18.16	21.21	24.72	28.47	32.16	35.45	38.01	39.48	39.63	38.30	35.48
9	19.50	19.90	20.13	20.15	19.97	19.72	19.55	19.65	20.18	21.24	22.90	25.11	27.77	30.68	33.57	36.15	38.09	39.08	38.91	37.43	34.67
9.5	24.62	26.05	26.99	27.39	27.31	26.89	26.34	25.87	25.68	25.92	26.68	27.97	29.71	31.73	33.81	35.67	37.03	37.61	37.21	35.71	33.16
10	30.05	32.30	33.75	34.35	34.19	33.45	32.37	31.20	30.18	29.51	29.31	29.62	30.40	31.52	32.79	33.98	34.81	35.06	34.55	33.19	31.01
10.5	35.41	38.29	40.05	40.69	40.30	39.11	37.39	35.43	33.51	31.87	30.66	29.98	29.80	30.04	30.54	31.11	31.51	31.54	31.06	29.99	28.38
11	40.44	43.74	45.65	46.17	45.43	43.69	41.24	38.43	35.57	32.94	30.73	29.06	27.96	27.37	27.18	27.22	27.30	27.25	26.94	26.34	25.51
11.5	44.91	48.46	50.37	50.64	49.45	47.06	43.84	40.15	36.35	32.74	29.57	26.97	25.03	23.70	22.93	22.56	22.46	22.48	22.54	22.60	22.76
12	48.67	52.31	54.07	53.99	52.27	49.21	45.20	40.64	35.94	31.42	27.36	23.94	21.25	19.32	18.10	17.49	17.38	17.66	18.26	19.21	20.57
12.5	51.62	55.19	56.70	56.20	53.91	50.16	45.39	40.02	34.49	29.17	24.35	20.24	16.98	14.61	13.11	12.44	12.51	13.26	14.63	16.67	19.48
13	53.69	57.08	58.26	57.30	54.43	50.03	44.57	38.49	32.27	26.29	20.87	16.26	12.62	10.01	8.46	7.94	8.42	9.85	12.23	15.60	20.08
13.5	54.92	58.01	58.81	57.37	53.97	49.01	42.97	36.34	29.60	23.16	17.36	12.47	8.66	6.05	4.69	4.57	5.70	8.07	11.69	16.63	23.03
14	55.38	58.11	58.49	56.61	52.77	47.38	40.93	33.94	26.89	20.22	14.29	9.38	5.68	3.33	2.42	2.97	5.02	8.57	13.69	20.45	29.00
14.5	55.32	57.60	57.58	55.34	51.17	45.52	38.87	31.75	24.66	18.03	12.24	7.59	4.28	2.49	2.31	3.82	7.05	12.07	18.93	27.76	38.71
15	55.10	56.91	56.50	53.99	49.66	43.94	37.33	30.35	23.49	17.20	11.85	7.76	5.15	4.21	5.07	7.81	12.51	19.26	28.14	39.28	52.85

Table 32 - Radial basis functions determined by *MATLAB*® for monounsaturates nitrates vs time; where nitrates amount 5 means 0.125 g/l, 10 = 0.250 g/l and 15 = 0.375g/l (Figure 3.10c)

Monounsaturates / %	**Nitrates**																				
Time / days	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
3	40.42	46.17	48.54	47.74	44.20	38.53	31.41	23.56	15.66	8.31	2.07	-2.64	-5.44	-6.05	-4.24	0.18	7.35	17.42	30.54	46.85	66.55
3.2	46.48	52.02	54.12	52.99	49.06	42.92	35.26	26.77	18.14	9.95	2.75	-3.06	-7.12	-9.17	-9.00	-6.46	-1.41	6.25	16.66	29.96	46.32
3.4	50.77	56.12	58.08	56.84	52.80	46.55	38.73	30.03	21.10	12.52	4.80	-1.67	-6.55	-9.62	-10.69	-9.63	-6.34	-0.74	7.28	17.85	31.14
3.6	53.11	58.28	60.20	59.02	55.14	49.08	41.46	32.94	24.13	15.58	7.77	1.07	-4.21	-7.88	-9.79	-9.84	-7.95	-4.07	1.89	10.02	20.49
3.8	53.53	58.51	60.45	59.48	55.95	50.35	43.24	35.23	26.91	18.77	11.27	4.74	-0.55	-4.43	-6.79	-7.57	-6.73	-4.23	-0.03	5.96	13.89
4	52.22	56.96	58.95	58.29	55.26	50.31	43.96	36.75	29.22	21.83	14.99	8.99	4.06	0.33	-2.13	-3.29	-3.15	-1.72	1.03	5.16	10.81
4.2	49.43	53.89	55.92	55.60	53.17	49.01	43.59	37.39	30.91	24.55	18.66	13.50	9.25	6.01	3.79	2.58	2.33	3.00	4.59	7.13	10.75
4.4	45.49	49.57	51.61	51.62	49.82	46.53	42.16	37.14	31.89	26.77	22.08	18.03	14.74	12.27	10.58	9.61	9.27	9.47	10.17	11.39	13.23
4.6	40.72	44.33	46.28	46.59	45.40	43.01	39.75	35.99	32.10	28.38	25.08	22.34	20.25	18.79	17.89	17.43	17.26	17.25	17.32	17.46	17.75
4.8	35.46	38.46	40.23	40.75	40.12	38.59	36.44	33.99	31.53	29.31	27.52	26.26	25.54	25.30	25.40	25.66	25.90	25.92	25.59	24.88	23.83
5	30.05	32.30	33.75	34.35	34.19	33.45	32.37	31.20	30.18	29.51	29.31	29.62	30.40	31.52	32.79	33.98	34.81	35.06	34.55	33.19	31.01
5.2	24.84	26.16	27.13	27.67	27.84	27.78	27.68	27.72	28.11	28.97	30.37	32.29	34.64	37.23	39.79	42.03	43.63	44.29	43.76	41.94	38.82
5.4	20.19	20.40	20.70	21.01	21.34	21.81	22.54	23.68	25.37	27.69	30.64	34.17	38.10	42.19	46.12	49.51	51.99	53.18	52.80	50.68	46.79
5.6	16.52	15.40	14.82	14.68	14.98	15.77	17.15	19.22	22.07	25.72	30.13	35.17	40.63	46.21	51.51	56.10	59.53	61.35	61.23	58.93	54.42
5.8	14.23	11.56	9.87	9.05	9.07	9.96	11.77	14.55	18.35	23.14	28.83	35.25	42.13	49.10	55.72	61.49	65.88	68.38	68.59	66.23	61.20
6	13.78	9.32	6.27	4.51	3.99	4.70	6.66	9.89	14.38	20.07	26.81	34.40	42.51	50.72	58.54	65.39	70.69	73.86	74.43	72.06	66.63
6.2	15.66	9.14	4.45	1.47	0.11	0.34	2.16	5.52	10.40	16.67	24.18	32.66	41.74	50.96	59.77	67.54	73.63	77.40	78.30	75.95	70.19
6.4	20.34	11.50	4.90	0.39	-2.13	-2.70	-1.39	1.76	6.66	13.18	21.09	30.11	39.84	49.76	59.29	67.74	74.43	78.64	79.79	77.44	71.41
6.6	28.35	16.91	8.09	1.73	-2.27	-4.02	-3.57	-1.02	3.51	9.86	17.79	26.95	36.92	47.17	57.07	65.89	72.90	77.36	78.62	76.22	69.93
6.8	40.19	25.88	14.54	6.01	0.16	-3.13	-3.94	-2.40	1.34	7.09	14.58	23.44	33.22	43.37	53.22	62.03	69.04	73.47	74.65	72.09	65.56
7	56.40	38.93	24.77	13.73	5.68	0.47	-2.01	-1.90	0.61	5.30	11.88	19.96	29.08	38.65	48.00	56.38	63.01	67.10	67.98	65.14	58.34

Table 33 - Radial basis functions determined by *MATLAB*® for monounsaturates iron vs time; where iron amount 5 means 2.49 mg/l, 10 = 4.98 mg/l and 15 = 7.47 mg/l (Figure 3.10d)

Monounsaturates / %	**Iron**																				
Time / days	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
3	41.78	25.96	14.98	7.93	3.92	2.11	1.71	2.01	2.43	2.54	2.07	0.93	-0.78	-2.78	-4.62	-5.71	-5.34	-2.71	3.04	12.80	27.48
3.2	27.56	14.25	5.62	0.74	-1.31	-1.41	-0.37	1.09	2.36	3.01	2.75	1.48	-0.67	-3.44	-6.35	-8.79	-10.04	-9.27	-5.59	1.92	14.20
3.4	16.07	5.06	-1.43	-4.34	-4.61	-3.16	-0.81	1.70	3.74	4.87	4.80	3.43	0.88	-2.57	-6.43	-10.09	-12.80	-13.70	-11.89	-6.41	3.68
3.6	6.91	-2.04	-6.60	-7.74	-6.43	-3.59	-0.07	3.37	6.10	7.67	7.77	6.32	3.42	-0.62	-5.33	-10.06	-14.05	-16.44	-16.29	-12.63	-4.49
3.8	-0.31	-7.43	-10.28	-9.86	-7.15	-3.09	1.46	5.72	9.06	11.01	11.27	9.75	6.56	2.01	-3.42	-9.08	-14.19	-17.87	-19.17	-17.11	-10.68
4	-5.90	-11.43	-12.80	-11.02	-7.10	-1.98	3.45	8.42	12.29	14.58	14.99	13.42	9.99	5.01	-1.03	-7.48	-13.54	-18.32	-20.87	-20.17	-15.21
4.2	-10.15	-14.33	-14.44	-11.51	-6.56	-0.55	5.64	11.22	15.54	18.12	18.66	17.06	13.45	8.12	1.58	-5.52	-12.36	-18.06	-21.64	-22.08	-18.35
4.4	-13.29	-16.35	-15.43	-11.56	-5.76	1.00	7.81	13.89	18.59	21.42	22.08	20.47	16.71	11.12	4.19	-3.42	-10.89	-17.32	-21.73	-23.08	-20.33
4.6	-15.50	-17.70	-15.96	-11.35	-4.88	2.46	9.77	16.26	21.27	24.31	25.08	23.48	19.63	13.84	6.62	-1.36	-9.30	-16.28	-21.31	-23.34	-21.34
4.8	-16.94	-18.51	-16.19	-11.03	-4.07	3.71	11.40	18.20	23.45	26.65	27.52	25.95	22.05	16.15	8.75	0.51	-7.74	-15.08	-20.52	-23.02	-21.51
5	-17.71	-18.90	-16.21	-10.71	-3.43	4.64	12.58	19.59	25.01	28.35	29.31	27.78	23.89	17.94	10.45	2.10	-6.30	-13.83	-19.48	-22.21	-20.95
5.2	-17.88	-18.92	-16.09	-10.45	-3.03	5.17	13.24	20.37	25.90	29.33	30.37	28.90	25.05	19.14	11.67	3.33	-5.07	-12.60	-18.25	-20.98	-19.72
5.4	-17.46	-18.62	-15.88	-10.29	-2.92	5.27	13.34	20.49	26.06	29.54	30.64	29.26	25.51	19.70	12.36	4.16	-4.07	-11.42	-16.86	-19.37	-17.86
5.6	-16.45	-17.97	-15.54	-10.23	-3.08	4.92	12.87	19.94	25.47	28.97	30.13	28.84	25.23	19.62	12.51	4.59	-3.31	-10.28	-15.31	-17.35	-15.33
5.8	-14.77	-16.91	-15.04	-10.22	-3.49	4.18	11.86	18.74	24.17	27.63	28.83	27.67	24.25	18.91	12.15	4.65	-2.76	-9.15	-13.54	-14.87	-12.09
6	-12.33	-15.34	-14.27	-10.16	-4.04	3.11	10.38	16.97	22.20	25.59	26.81	25.79	22.62	17.63	11.34	4.41	-2.32	-7.94	-11.46	-11.84	-8.03
6.2	-8.95	-13.11	-13.09	-9.92	-4.62	1.84	8.56	14.72	19.68	22.94	24.18	23.31	20.45	15.91	10.21	4.01	-1.87	-6.50	-8.92	-8.10	-3.00
6.4	-4.44	-10.00	-11.28	-9.28	-5.01	0.57	6.57	12.19	16.78	19.85	21.09	20.41	17.91	13.91	8.92	3.62	-1.21	-4.65	-5.73	-3.45	3.21
6.6	1.48	-5.76	-8.59	-8.00	-4.97	-0.46	4.66	9.61	13.74	16.57	17.79	17.30	15.23	11.87	7.74	3.49	-0.10	-2.12	-1.63	2.36	10.86
6.8	9.13	-0.05	-4.68	-5.73	-4.17	-0.92	3.16	7.30	10.87	13.40	14.58	14.32	12.73	10.12	6.97	3.94	1.79	1.41	3.71	9.67	20.26
7	18.89	7.52	0.84	-2.08	-2.20	-0.40	2.47	5.68	8.60	10.76	11.88	11.87	10.83	9.06	7.03	5.39	4.87	6.33	10.68	18.85	31.79

Table 34 - Radial basis functions determined by *MATLAB*® for monounsaturates iron vs phosphates; where iron amount 5 means 2.49 mg/l, 10 = 4.98 mg/l and 15 = 7.47 mg/l; where phosphate amount 5 means a ratio of K_2HPO_4:KH_2PO_4 of 0.0375:0.0875 g/l, 10 = 0.0750:0.1750 g/l and 15 = 0.1125:0.2625 g/l (Figure 3.10e)

Monounsaturates / %	**Iron**																				
Phosphates	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
5	42.23	24.55	11.76	3.00	-2.56	-5.70	-7.15	-7.54	-7.41	-7.14	-6.98	-7.01	-7.13	-7.11	-6.54	-4.88	-1.50	4.33	13.42	26.58	44.69
5.5	24.88	9.96	-0.25	-6.62	-10.02	-11.27	-11.13	-10.26	-9.22	-8.41	-8.09	-8.34	-9.06	-10.00	-10.74	-10.73	-9.29	-5.66	0.99	11.53	26.85
6	11.33	-1.03	-8.82	-12.97	-14.37	-13.87	-12.26	-10.22	-8.34	-7.03	-6.57	-7.02	-8.30	-10.15	-12.11	-13.63	-13.99	-12.40	-8.02	0.07	12.79
6.5	1.03	-8.99	-14.58	-16.69	-16.26	-14.16	-11.21	-8.11	-5.46	-3.70	-3.10	-3.75	-5.55	-8.22	-11.31	-14.23	-16.24	-16.54	-14.23	-8.38	1.95
7	-6.56	-14.46	-18.08	-18.37	-16.29	-12.76	-8.60	-4.55	-1.21	0.95	1.66	0.83	-1.44	-4.86	-8.96	-13.14	-16.65	-18.64	-18.20	-14.38	-6.19
7.5	-11.90	-17.96	-19.83	-18.52	-15.00	-10.21	-5.00	-0.11	3.81	6.32	7.13	6.14	3.45	-0.63	-5.64	-10.93	-15.75	-19.24	-20.45	-18.40	-12.10
8	-15.43	-19.90	-20.30	-17.63	-12.89	-7.02	-0.91	4.67	9.10	11.90	12.78	11.64	8.60	3.94	-1.84	-8.09	-14.04	-18.81	-21.43	-20.90	-16.21
8.5	-17.50	-20.69	-19.88	-16.10	-10.36	-3.62	3.21	9.37	14.20	17.23	18.16	16.90	13.55	8.41	2.00	-5.05	-11.93	-17.75	-21.53	-22.25	-18.88
9	-18.42	-20.63	-18.90	-14.27	-7.77	-0.37	7.01	13.60	18.74	21.94	22.90	21.52	17.93	12.42	5.50	-2.16	-9.77	-16.40	-21.08	-22.77	-20.42
9.5	-18.44	-19.98	-17.61	-12.41	-5.39	2.45	10.20	17.08	22.41	25.71	26.68	25.22	21.44	15.66	8.39	0.28	-7.83	-15.04	-20.34	-22.70	-21.06
10	-17.71	-18.90	-16.21	-10.71	-3.43	4.64	12.58	19.59	25.01	28.35	29.31	27.78	23.89	17.94	10.45	2.10	-6.30	-13.83	-19.48	-22.21	-20.95
10.5	-16.33	-17.49	-14.78	-9.26	-1.97	6.10	14.03	21.03	26.43	29.74	30.66	29.09	25.15	19.15	11.60	3.18	-5.29	-12.88	-18.60	-21.39	-20.18
11	-14.32	-15.77	-13.35	-8.10	-1.06	6.79	14.53	21.37	26.65	29.86	30.73	29.14	25.21	19.26	11.80	3.51	-4.80	-12.20	-17.70	-20.24	-18.77
11.5	-11.61	-13.69	-11.86	-7.15	-0.61	6.80	14.16	20.69	25.72	28.78	29.57	27.98	24.14	18.35	11.14	3.16	-4.77	-11.73	-16.72	-18.71	-16.65
12	-8.09	-11.11	-10.16	-6.28	-0.48	6.26	13.06	19.14	23.83	26.67	27.36	25.80	22.10	16.59	9.77	2.29	-5.03	-11.29	-15.51	-16.65	-13.67
12.5	-3.54	-7.81	-8.04	-5.25	-0.44	5.43	11.49	16.96	21.22	23.78	24.35	22.84	19.36	14.23	7.94	1.15	-5.35	-10.67	-13.83	-13.82	-9.62
13	2.29	-3.52	-5.20	-3.77	-0.18	4.61	9.76	14.50	18.22	20.44	20.87	19.44	16.25	11.61	6.00	0.07	-5.40	-9.54	-11.38	-9.95	-4.22
13.5	9.76	2.12	-1.29	-1.45	0.68	4.21	8.28	12.16	15.24	17.07	17.36	16.03	13.18	9.13	4.34	-0.56	-4.79	-7.51	-7.79	-4.65	2.89
14	19.25	9.51	4.12	2.13	2.61	4.68	7.53	10.42	12.78	14.16	14.29	13.09	10.66	7.29	3.45	-0.24	-3.05	-4.14	-2.61	2.49	12.11
14.5	31.22	19.14	11.54	7.50	6.13	6.58	8.04	9.84	11.40	12.29	12.24	11.19	9.23	6.64	3.88	1.55	0.34	1.09	4.66	11.96	23.92
15	46.19	31.53	21.50	15.22	11.83	10.49	10.45	11.04	11.72	12.07	11.85	10.97	9.54	7.82	6.26	5.42	6.00	8.77	14.57	24.30	38.84

Table 35 – Radial basis functions determined by *MATLAB*® for monounsaturates phosphates vs time; where phosphate amount 5 means a ratio of K_2HPO_4:KH_2PO_4 of 0.0375:0.0875 g/l, 10 = 0.0750:0.1750 g/l and 15 = 0.1125:0.2625 g/l (Figure 3.10f)

Monounsaturates / %	**Phosphates**																				
Time / days	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
3	21.21	9.59	1.77	-2.93	-5.17	-5.58	-4.74	-3.16	-1.30	0.53	2.07	3.22	3.99	4.51	5.03	5.90	7.56	10.50	15.29	22.53	32.86
3.2	10.73	1.08	-4.95	-8.05	-8.89	-8.08	-6.21	-3.81	-1.30	0.96	2.75	3.94	4.56	4.72	4.67	4.75	5.40	7.11	10.44	16.00	24.43
3.4	3.05	-4.86	-9.31	-11.00	-10.61	-8.75	-6.01	-2.90	0.15	2.79	4.80	6.05	6.56	6.43	5.92	5.35	5.13	5.78	7.83	11.90	18.63
3.6	-2.32	-8.69	-11.77	-12.25	-10.81	-8.07	-4.59	-0.89	2.61	5.57	7.77	9.08	9.51	9.17	8.28	7.18	6.25	5.99	6.94	9.71	14.94
3.8	-5.83	-10.87	-12.77	-12.23	-9.91	-6.43	-2.36	1.82	5.69	8.91	11.27	12.64	13.02	12.52	11.34	9.80	8.29	7.27	7.29	8.94	12.86
4	-7.86	-11.78	-12.70	-11.31	-8.28	-4.21	0.35	4.92	9.07	12.50	14.99	16.41	16.75	16.13	14.72	12.84	10.84	9.20	8.43	9.12	11.92
4.2	-8.80	-11.79	-11.90	-9.83	-6.22	-1.68	3.26	8.11	12.49	16.08	18.66	20.11	20.43	19.71	18.12	15.95	13.56	11.40	9.97	9.86	11.71
4.4	-8.95	-11.20	-10.67	-8.06	-4.00	0.90	6.12	11.20	15.74	19.43	22.08	23.55	23.84	23.02	21.27	18.87	16.15	13.56	11.59	10.81	11.87
4.6	-8.57	-10.26	-9.25	-6.23	-1.84	3.33	8.76	13.99	18.64	22.40	25.08	26.54	26.78	25.86	23.97	21.35	18.35	15.40	12.98	11.67	12.09
4.8	-7.86	-9.18	-7.84	-4.54	0.09	5.45	11.02	16.35	21.06	24.85	27.52	28.95	29.11	28.08	26.04	23.22	19.98	16.72	13.93	12.20	12.14
5	-6.98	-8.09	-6.57	-3.10	1.66	7.13	12.78	18.16	22.90	26.68	29.31	30.66	30.73	29.57	27.36	24.35	20.87	17.36	14.29	12.24	11.85
5.2	-6.00	-7.08	-5.51	-2.00	2.80	8.30	13.97	19.36	24.07	27.81	30.37	31.62	31.55	30.24	27.85	24.64	20.97	17.24	13.97	11.72	11.13
5.4	-4.94	-6.15	-4.69	-1.27	3.47	8.93	14.56	19.90	24.54	28.20	30.64	31.76	31.53	30.05	27.47	24.08	20.23	16.35	12.95	10.61	9.99
5.6	-3.72	-5.27	-4.08	-0.86	3.71	9.02	14.54	19.76	24.30	27.83	30.13	31.08	30.68	29.00	26.24	22.69	18.70	14.73	11.30	9.00	8.49
5.8	-2.25	-4.31	-3.56	-0.71	3.56	8.64	13.94	18.99	23.35	26.71	28.83	29.60	29.00	27.14	24.22	20.53	16.46	12.49	9.13	7.01	6.79
6	-0.32	-3.12	-2.99	-0.67	3.16	7.87	12.87	17.65	21.77	24.91	26.81	27.38	26.59	24.56	21.50	17.74	13.68	9.80	6.66	4.88	5.12
6.2	2.30	-1.46	-2.16	-0.55	2.68	6.88	11.45	15.86	19.67	22.52	24.18	24.53	23.56	21.39	18.26	14.51	10.55	6.90	4.13	2.85	3.77
6.4	5.89	0.95	-0.80	-0.10	2.34	5.87	9.87	13.80	17.20	19.72	21.09	21.22	20.09	17.84	14.71	11.07	7.35	4.08	1.85	1.28	3.08
6.6	10.81	4.45	1.42	0.99	2.45	5.13	8.40	11.72	14.62	16.72	17.79	17.69	16.43	14.17	11.15	7.75	4.42	1.70	0.20	0.55	3.45
6.8	17.46	9.42	4.87	3.08	3.35	4.99	7.37	9.95	12.24	13.86	14.58	14.26	12.93	10.73	7.93	4.91	2.14	0.17	-0.39	1.10	5.34
7	26.26	16.30	10.00	6.63	5.48	5.88	7.21	8.89	10.46	11.54	11.88	11.36	10.00	7.96	5.50	3.02	0.99	-0.03	0.56	3.42	9.22

Table 36 - Radial basis functions determined by *MATLAB*® for monounsaturates mass nitrates vs iron; where nitrates amount 5 means 0.125 g/l, 10 = 0.250 g/l and 15 = 0.375g/l; where iron amount 5 means 2.49 mg/l, 10 = 4.98 mg/l and 15 = 7.47 mg/l (Figure 3.11a)

Monounsaturates / mg	**Nitrates**																				
Iron	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
5	21.88	23.51	24.98	26.26	27.33	28.16	28.73	28.99	28.95	28.59	27.89	26.88	25.55	23.94	22.08	20.01	17.76	15.40	12.96	10.49	8.03
5.5	23.29	25.05	26.66	28.08	29.30	30.26	30.95	31.34	31.40	31.12	30.49	29.51	28.20	26.59	24.70	22.58	20.28	17.84	15.32	12.76	10.21
6	24.57	26.47	28.22	29.79	31.15	32.26	33.09	33.60	33.77	33.58	33.01	32.09	30.80	29.19	27.28	25.13	22.77	20.27	17.67	15.03	12.37
6.5	25.72	27.75	29.64	31.36	32.87	34.13	35.09	35.74	36.02	35.92	35.44	34.56	33.31	31.70	29.78	27.60	25.19	22.63	19.96	17.23	14.49
7	26.73	28.88	30.91	32.78	34.43	35.84	36.94	37.72	38.12	38.12	37.71	36.89	35.67	34.08	32.15	29.94	27.49	24.87	22.14	19.33	16.51
7.5	27.59	29.86	32.02	34.02	35.82	37.36	38.60	39.50	40.02	40.12	39.79	39.02	37.84	36.26	34.33	32.09	29.61	26.94	24.15	21.27	18.37
8	28.29	30.67	32.95	35.07	37.00	38.67	40.04	41.06	41.68	41.87	41.61	40.90	39.75	38.18	36.25	34.00	31.49	28.78	25.93	23.00	20.03
8.5	28.83	31.31	33.68	35.92	37.95	39.74	41.22	42.34	43.05	43.33	43.13	42.47	41.35	39.80	37.87	35.61	33.07	30.33	27.44	24.46	21.43
9	29.21	31.75	34.21	36.53	38.66	40.54	42.11	43.31	44.11	44.45	44.31	43.69	42.60	41.06	39.13	36.86	34.31	31.55	28.62	25.60	22.54
9.5	29.41	32.01	34.53	36.91	39.10	41.05	42.68	43.95	44.80	45.19	45.10	44.51	43.44	41.93	40.00	37.73	35.17	32.39	29.45	26.41	23.31
10	29.44	32.07	34.62	37.04	39.27	41.26	42.93	44.24	45.12	45.55	45.48	44.92	43.87	42.36	40.45	38.18	35.62	32.84	29.89	26.84	23.74
10.5	29.31	31.94	34.50	36.93	39.17	41.16	42.85	44.16	45.06	45.50	45.44	44.89	43.85	42.36	40.46	38.20	35.65	32.88	29.94	26.90	23.80
11	29.00	31.62	34.16	36.57	38.79	40.76	42.43	43.74	44.62	45.05	44.99	44.44	43.41	41.93	40.04	37.80	35.27	32.52	29.60	26.58	23.51
11.5	28.53	31.11	33.61	35.97	38.15	40.08	41.71	42.97	43.83	44.23	44.15	43.60	42.57	41.09	39.22	37.00	34.50	31.78	28.90	25.92	22.88
12	27.91	30.43	32.86	35.16	37.27	39.13	40.69	41.90	42.70	43.06	42.96	42.39	41.35	39.89	38.04	35.85	33.38	30.70	27.86	24.93	21.95
12.5	27.14	29.59	31.94	34.15	36.17	37.95	39.42	40.55	41.29	41.60	41.46	40.86	39.82	38.36	36.53	34.37	31.94	29.31	26.53	23.66	20.74
13	26.24	28.59	30.84	32.95	34.87	36.55	37.93	38.97	39.63	39.88	39.69	39.07	38.02	36.57	34.76	32.63	30.25	27.67	24.96	22.15	19.32
13.5	25.21	27.46	29.60	31.60	33.41	34.97	36.25	37.20	37.78	37.96	37.72	37.07	36.00	34.56	32.77	30.69	28.36	25.84	23.19	20.47	17.72
14	24.06	26.20	28.23	30.12	31.80	33.25	34.42	35.27	35.76	35.87	35.58	34.90	33.83	32.39	30.63	28.59	26.31	23.86	21.29	18.65	15.99
14.5	22.82	24.84	26.75	28.51	30.08	31.41	32.47	33.21	33.62	33.66	33.33	32.62	31.54	30.12	28.39	26.39	24.18	21.80	19.31	16.76	14.19
15	21.48	23.39	25.18	26.81	28.26	29.47	30.42	31.07	31.39	31.37	30.99	30.26	29.18	27.77	26.08	24.13	21.99	19.69	17.28	14.83	12.36

Table 37 - Radial basis functions determined by *MATLAB*® for monounsaturates mass nitrates vs phosphates; where nitrates amount 5 means 0.125 g/l, 10 = 0.250 g/l and 15 = 0.375g/l; where phosphates of K_2HPO_4:KH_2PO_4 of 0.0375:0.0875 g/l, 10 = 0.0750:0.1750 g/l and 15 = 0.1125:0.2625 g/l (Figure 3.11b)

Monounsaturates / mg	**Nitrates**																				
Phosphates	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
5	16.62	19.48	22.43	25.45	28.49	31.50	34.44	37.26	39.92	42.36	44.56	46.49	48.11	49.41	50.37	50.96	51.18	51.02	50.47	49.52	48.19
5.5	18.18	21.07	24.04	27.06	30.09	33.07	35.96	38.70	41.25	43.56	45.60	47.34	48.76	49.84	50.55	50.90	50.88	50.48	49.69	48.53	47.01
6	19.80	22.70	25.68	28.70	31.69	34.62	37.44	40.08	42.50	44.65	46.51	48.03	49.21	50.03	50.47	50.55	50.25	49.58	48.54	47.16	45.43
6.5	21.42	24.34	27.32	30.31	33.26	36.13	38.84	41.36	43.63	45.60	47.25	48.53	49.44	49.98	50.13	49.90	49.30	48.34	47.04	45.42	43.49
7	23.02	25.95	28.91	31.86	34.75	37.53	40.14	42.52	44.62	46.39	47.80	48.82	49.45	49.68	49.51	48.96	48.05	46.80	45.22	43.36	41.22
7.5	24.55	27.46	30.39	33.29	36.11	38.79	41.28	43.50	45.41	46.97	48.13	48.88	49.21	49.12	48.63	47.75	46.53	44.97	43.12	41.01	38.68
8	25.95	28.84	31.72	34.56	37.29	39.86	42.20	44.26	45.97	47.30	48.21	48.68	48.71	48.30	47.49	46.29	44.75	42.90	40.79	38.45	35.92
8.5	27.18	30.03	32.85	35.61	38.24	40.68	42.87	44.75	46.26	47.36	48.01	48.20	47.93	47.22	46.09	44.58	42.74	40.62	38.26	35.70	32.99
9	28.19	30.99	33.74	36.40	38.91	41.21	43.23	44.93	46.23	47.10	47.50	47.42	46.87	45.87	44.44	42.65	40.54	38.16	35.57	32.82	29.96
9.5	28.96	31.68	34.34	36.88	39.26	41.41	43.26	44.76	45.86	46.50	46.66	46.33	45.52	44.24	42.56	40.51	38.15	35.56	32.77	29.86	26.86
10	29.44	32.07	34.62	37.04	39.27	41.26	42.93	44.24	45.12	45.55	45.48	44.92	43.87	42.36	40.45	38.18	35.62	32.84	29.89	26.84	23.74
10.5	29.64	32.16	34.58	36.86	38.93	40.74	42.23	43.34	44.02	44.24	43.96	43.19	41.93	40.23	38.12	35.68	32.96	30.03	26.96	23.81	20.63
11	29.53	31.93	34.21	36.33	38.23	39.86	41.16	42.08	42.57	42.59	42.12	41.16	39.73	37.87	35.61	33.03	30.19	27.16	24.01	20.79	17.56
11.5	29.12	31.38	33.51	35.47	37.19	38.64	39.75	40.48	40.78	40.62	39.98	38.87	37.30	35.31	32.95	30.27	27.35	24.26	21.06	17.81	14.56
12	28.42	30.53	32.50	34.29	35.83	37.09	38.02	38.56	38.69	38.37	37.59	36.34	34.67	32.58	30.15	27.42	24.46	21.34	18.12	14.87	11.63
12.5	27.45	29.41	31.21	32.82	34.18	35.26	36.01	36.38	36.34	35.88	34.97	33.63	31.87	29.73	27.25	24.50	21.54	18.43	15.23	11.99	8.78
13	26.22	28.02	29.66	31.09	32.28	33.18	33.76	33.97	33.79	33.20	32.19	30.77	28.95	26.78	24.30	21.55	18.61	15.53	12.37	9.19	6.02
13.5	24.77	26.41	27.88	29.14	30.16	30.89	31.31	31.38	31.07	30.37	29.28	27.80	25.95	23.77	21.30	18.60	15.70	12.68	9.58	6.46	3.36
14	23.12	24.61	25.91	27.01	27.86	28.44	28.71	28.65	28.23	27.44	26.28	24.77	22.91	20.74	18.31	15.65	12.82	9.87	6.85	3.81	0.79
14.5	21.31	22.64	23.79	24.73	25.43	25.86	26.00	25.82	25.30	24.44	23.24	21.70	19.85	17.71	15.32	12.73	9.98	7.11	4.18	1.23	-1.70
15	19.35	20.53	21.53	22.33	22.89	23.19	23.21	22.93	22.33	21.41	20.18	18.63	16.80	14.70	12.37	9.85	7.19	4.42	1.59	-1.26	-4.10

Table 38 - Radial basis functions determined by *MATLAB*® for monounsaturates mass nitrates vs time; where nitrates amount 5 means 0.125 g/l, 10 = 0.250 g/l and 15 = 0.375g/l (Figure 3.11c)

Monounsaturates / mg	**Nitrates**																				
Time / days	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
3	5.17	6.68	8.12	9.48	10.73	11.85	12.82	13.62	14.24	14.66	14.87	14.87	14.66	14.23	13.61	12.79	11.79	10.64	9.34	7.92	6.39
3.2	8.16	9.81	11.40	12.90	14.28	15.52	16.60	17.48	18.16	18.61	18.82	18.79	18.51	18.00	17.26	16.31	15.17	13.85	12.39	10.79	9.09
3.4	11.11	12.91	14.65	16.29	17.81	19.18	20.36	21.33	22.06	22.54	22.75	22.68	22.34	21.73	20.87	19.78	18.47	16.98	15.34	13.56	11.68
3.6	13.99	15.95	17.83	19.62	21.28	22.77	24.06	25.11	25.90	26.40	26.60	26.50	26.08	25.37	24.37	23.13	21.65	19.99	18.16	16.19	14.13
3.8	16.77	18.87	20.91	22.84	24.63	26.24	27.63	28.76	29.60	30.13	30.32	30.16	29.66	28.83	27.70	26.30	24.65	22.80	20.78	18.63	16.38
4	19.42	21.66	23.83	25.89	27.80	29.53	31.01	32.22	33.10	33.64	33.81	33.60	33.01	32.07	30.80	29.23	27.41	25.37	23.17	20.83	18.40
4.2	21.89	24.25	26.54	28.73	30.75	32.57	34.14	35.40	36.32	36.86	37.00	36.73	36.06	34.99	33.58	31.85	29.86	27.65	25.26	22.75	20.15
4.4	24.16	26.62	29.02	31.30	33.42	35.32	36.95	38.26	39.20	39.73	39.83	39.49	38.72	37.54	35.99	34.11	31.95	29.57	27.02	24.34	21.58
4.6	26.19	28.73	31.21	33.56	35.75	37.71	39.38	40.71	41.65	42.16	42.22	41.81	40.94	39.65	37.97	35.94	33.63	31.10	28.39	25.57	22.67
4.8	27.96	30.56	33.09	35.49	37.71	39.70	41.39	42.72	43.64	44.12	44.11	43.63	42.67	41.27	39.46	37.31	34.86	32.19	29.36	26.41	23.39
5	29.44	32.07	34.62	37.04	39.27	41.26	42.93	44.24	45.12	45.55	45.48	44.92	43.87	42.36	40.45	38.18	35.62	32.84	29.89	26.84	23.74
5.2	30.64	33.26	35.81	38.21	40.41	42.37	44.00	45.26	46.08	46.44	46.30	45.65	44.51	42.92	40.91	38.55	35.89	33.02	29.99	26.87	23.70
5.4	31.53	34.13	36.63	38.98	41.14	43.03	44.59	45.77	46.52	46.80	46.57	45.84	44.62	42.94	40.85	38.42	35.70	32.76	29.67	26.50	23.29
5.6	32.12	34.66	37.10	39.38	41.45	43.25	44.72	45.81	46.46	46.64	46.32	45.51	44.21	42.47	40.31	37.82	35.05	32.07	28.96	25.76	22.54
5.8	32.42	34.88	37.22	39.40	41.36	43.05	44.41	45.38	45.93	46.01	45.60	44.70	43.34	41.53	39.33	36.80	34.00	31.01	27.88	24.69	21.48
6	32.44	34.80	37.03	39.09	40.92	42.48	43.71	44.56	44.98	44.95	44.45	43.48	42.05	40.19	37.96	35.41	32.60	29.61	26.50	23.33	20.15
6.2	32.20	34.44	36.54	38.46	40.15	41.57	42.66	43.37	43.68	43.54	42.94	41.90	40.41	38.52	36.26	33.71	30.91	27.94	24.86	21.74	18.61
6.4	31.72	33.82	35.78	37.56	39.10	40.37	41.31	41.89	42.07	41.82	41.14	40.03	38.49	36.57	34.31	31.77	29.00	26.06	23.03	19.97	16.91
6.6	31.02	32.98	34.80	36.42	37.81	38.93	39.72	40.17	40.22	39.88	39.11	37.94	36.37	34.43	32.18	29.65	26.92	24.04	21.08	18.08	15.11
6.8	30.13	31.95	33.61	35.08	36.32	37.28	37.94	38.25	38.19	37.75	36.91	35.69	34.09	32.15	29.92	27.43	24.75	21.93	19.04	16.13	13.25
7	29.07	30.75	32.26	33.58	34.66	35.48	36.00	36.19	36.03	35.50	34.60	33.34	31.73	29.80	27.59	25.15	22.53	19.79	16.99	14.17	11.38

Table 39 - Radial basis functions determined by *MATLAB*® for monounsaturates mass iron vs time; where iron amount 5 means 2.49 mg/l, 10 = 4.98 mg/l and 15 = 7.47 mg/l (Figure 3.11d)

Monounsaturates / mg	**Iron**																				
Time / days	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
3	9.45	10.64	11.72	12.68	13.50	14.16	14.66	14.99	15.14	15.10	14.87	14.46	13.87	13.12	12.20	11.15	9.99	8.71	7.36	5.94	4.48
3.2	12.26	13.61	14.85	15.96	16.92	17.72	18.35	18.78	19.00	19.02	18.82	18.41	17.79	16.98	16.00	14.86	13.58	12.19	10.71	9.17	7.57
3.4	14.97	16.48	17.88	19.16	20.28	21.22	21.98	22.52	22.84	22.91	22.75	22.34	21.71	20.85	19.79	18.56	17.17	15.66	14.06	12.38	10.65
3.6	17.52	19.21	20.78	22.22	23.50	24.61	25.50	26.16	26.58	26.73	26.60	26.21	25.56	24.65	23.53	22.20	20.71	19.08	17.35	15.54	13.68
3.8	19.89	21.74	23.49	25.10	26.55	27.81	28.85	29.64	30.16	30.39	30.32	29.94	29.27	28.33	27.14	25.73	24.14	22.40	20.55	18.62	16.64
4	22.03	24.04	25.95	27.73	29.35	30.77	31.96	32.88	33.51	33.82	33.81	33.46	32.79	31.82	30.58	29.10	27.42	25.58	23.63	21.58	19.49
4.2	23.88	26.05	28.12	30.06	31.84	33.42	34.75	35.81	36.55	36.95	37.00	36.70	36.04	35.06	33.78	32.24	30.49	28.57	26.52	24.39	22.20
4.4	25.43	27.73	29.94	32.04	33.97	35.69	37.17	38.35	39.21	39.71	39.83	39.57	38.94	37.96	36.66	35.09	33.30	31.32	29.20	27.00	24.73
4.6	26.62	29.05	31.39	33.61	35.68	37.54	39.14	40.45	41.42	42.02	42.22	42.02	41.43	40.48	39.19	37.61	35.79	33.78	31.63	29.37	27.06
4.8	27.45	29.97	32.42	34.75	36.93	38.91	40.63	42.06	43.13	43.83	44.11	43.99	43.46	42.55	41.30	39.74	37.93	35.92	33.76	31.49	29.15
5	27.89	30.49	33.01	35.44	37.71	39.79	41.61	43.13	44.31	45.10	45.48	45.44	44.99	44.15	42.96	41.46	39.69	37.72	35.58	33.33	30.99
5.2	27.95	30.59	33.17	35.66	38.00	40.15	42.06	43.67	44.94	45.82	46.30	46.36	46.00	45.26	44.16	42.74	41.05	39.15	37.07	34.86	32.56
5.4	27.63	30.29	32.90	35.43	37.81	40.02	41.99	43.67	45.02	45.99	46.57	46.74	46.50	45.87	44.88	43.58	42.00	40.20	38.21	36.08	33.85
5.6	26.96	29.61	32.23	34.76	37.17	39.41	41.43	43.17	44.59	45.65	46.32	46.60	46.49	46.00	45.15	43.99	42.55	40.88	39.01	36.99	34.85
5.8	25.96	28.59	31.18	33.71	36.12	38.38	40.42	42.20	43.68	44.82	45.60	46.00	46.02	45.67	44.99	43.99	42.71	41.19	39.47	37.58	35.55
6	24.68	27.26	29.82	32.32	34.71	36.96	39.01	40.83	42.36	43.58	44.45	44.97	45.13	44.94	44.43	43.60	42.50	41.16	39.60	37.86	35.97
6.2	23.17	25.68	28.19	30.64	33.00	35.23	37.29	39.12	40.69	41.98	42.94	43.58	43.88	43.86	43.51	42.87	41.96	40.80	39.42	37.84	36.10
6.4	21.47	23.91	26.34	28.74	31.06	33.26	35.30	37.14	38.75	40.09	41.14	41.89	42.33	42.46	42.29	41.84	41.11	40.14	38.94	37.54	35.95
6.6	19.63	21.99	24.35	26.68	28.94	31.10	33.12	34.96	36.59	37.98	39.11	39.96	40.53	40.81	40.81	40.54	40.00	39.21	38.19	36.96	35.54
6.8	17.72	19.99	22.26	24.51	26.71	28.82	30.81	32.64	34.29	35.72	36.91	37.86	38.54	38.96	39.12	39.01	38.65	38.04	37.20	36.14	34.87
7	15.76	17.93	20.12	22.29	24.42	26.48	28.43	30.24	31.89	33.35	34.60	35.62	36.41	36.95	37.25	37.29	37.09	36.65	35.98	35.08	33.97

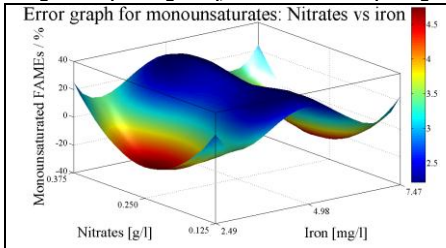
Table 40 - Radial basis functions determined by *MATLAB*® for monounsaturates mass iron vs phosphates; where iron amount 5 means 2.49 mg/l, 10 = 4.98 mg/l and 15 = 7.47 mg/l; where phosphate amount 5 means a ratio of K_2HPO_4:KH_2PO_4 of 0.0375:0.0875 g/l, 10 = 0.0750:0.1750 g/l and 15 = 0.1125:0.2625 g/l (Figure 3.11e)

Monounsaturates / mg	**Iron**																				
Phosphates	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
5	38.61	40.22	41.63	42.82	43.78	44.50	44.99	45.24	45.25	45.03	44.56	43.87	42.97	41.85	40.56	39.09	37.48	35.74	33.90	31.98	29.99
5.5	38.49	40.23	41.76	43.09	44.18	45.04	45.66	46.03	46.14	46.00	45.60	44.96	44.07	42.96	41.65	40.15	38.49	36.70	34.79	32.80	30.74
6	38.13	39.99	41.66	43.13	44.38	45.38	46.14	46.64	46.87	46.83	46.51	45.91	45.06	43.95	42.63	41.10	39.40	37.55	35.58	33.52	31.40
6.5	37.54	39.52	41.33	42.95	44.35	45.52	46.43	47.07	47.42	47.48	47.25	46.71	45.89	44.80	43.46	41.91	40.17	38.27	36.24	34.12	31.93
7	36.72	38.82	40.77	42.54	44.10	45.43	46.49	47.28	47.77	47.94	47.80	47.33	46.55	45.47	44.13	42.56	40.78	38.83	36.76	34.58	32.33
7.5	35.68	37.91	39.99	41.90	43.62	45.10	46.33	47.27	47.89	48.18	48.13	47.73	47.00	45.94	44.61	43.01	41.21	39.22	37.10	34.88	32.58
8	34.45	36.78	38.99	41.04	42.91	44.54	45.92	47.01	47.76	48.17	48.21	47.88	47.20	46.18	44.85	43.25	41.42	39.41	37.25	34.99	32.66
8.5	33.04	35.47	37.78	39.96	41.96	43.74	45.26	46.47	47.36	47.87	48.01	47.76	47.13	46.14	44.83	43.23	41.40	39.37	37.19	34.91	32.55
9	31.47	33.97	36.38	38.66	40.77	42.68	44.32	45.66	46.66	47.28	47.50	47.32	46.75	45.80	44.52	42.94	41.11	39.08	36.90	34.61	32.24
9.5	29.75	32.30	34.78	37.15	39.36	41.36	43.10	44.55	45.64	46.35	46.66	46.55	46.04	45.14	43.90	42.35	40.55	38.54	36.37	34.08	31.72
10	27.89	30.49	33.01	35.44	37.71	39.79	41.61	43.13	44.31	45.10	45.48	45.44	44.99	44.15	42.96	41.46	39.69	37.72	35.58	33.33	30.99
10.5	25.93	28.53	31.08	33.54	35.85	37.97	39.85	41.43	42.66	43.52	43.96	43.99	43.60	42.83	41.70	40.26	38.55	36.63	34.55	32.34	30.05
11	23.86	26.46	29.01	31.47	33.79	35.93	37.83	39.44	40.72	41.62	42.12	42.21	41.89	41.19	40.13	38.76	37.13	35.28	33.27	31.12	28.89
11.5	21.71	24.28	26.80	29.24	31.55	33.68	35.59	37.21	38.51	39.44	39.98	40.13	39.87	39.25	38.27	36.99	35.45	33.69	31.75	29.69	27.54
12	19.49	22.01	24.49	26.89	29.16	31.26	33.15	34.76	36.06	37.01	37.59	37.78	37.59	37.04	36.15	34.97	33.52	31.86	30.03	28.06	26.00
12.5	17.22	19.67	22.09	24.42	26.64	28.70	30.54	32.12	33.41	34.37	34.97	35.21	35.08	34.61	33.81	32.73	31.39	29.84	28.11	26.26	24.30
13	14.90	17.28	19.62	21.88	24.03	26.02	27.81	29.35	30.62	31.57	32.19	32.46	32.40	32.00	31.29	30.31	29.08	27.64	26.03	24.29	22.44
13.5	12.56	14.85	17.10	19.28	21.34	23.26	24.98	26.48	27.71	28.65	29.28	29.58	29.57	29.25	28.63	27.75	26.63	25.31	23.82	22.19	20.46
14	10.20	12.40	14.56	16.64	18.62	20.45	22.10	23.54	24.73	25.65	26.28	26.62	26.65	26.39	25.86	25.08	24.07	22.86	21.49	19.98	18.36
14.5	7.84	9.94	12.00	13.98	15.87	17.61	19.19	20.56	21.71	22.61	23.24	23.59	23.67	23.47	23.02	22.33	21.43	20.33	19.08	17.69	16.18
15	5.48	7.48	9.44	11.33	13.11	14.77	16.27	17.58	18.68	19.55	20.18	20.55	20.66	20.52	20.14	19.54	18.74	17.75	16.61	15.33	13.94

Table 41 – Radial basis functions determined by *MATLAB*® for monounsaturates mass phosphates vs time; where phosphate amount 5 means a ratio of K_2HPO_4:KH_2PO_4 of 0.0375:0.0875 g/l, 10 = 0.0750:0.1750 g/l and 15 = 0.1125:0.2625 g/l (Figure 3.11f)

Monounsaturates / mg	**Phosphates**																				
Time / days	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
3	8.43	9.29	10.20	11.12	12.03	12.87	13.61	14.21	14.64	14.87	14.87	14.65	14.18	13.49	12.57	11.45	10.14	8.67	7.06	5.33	3.50
3.2	12.52	13.44	14.39	15.34	16.26	17.10	17.82	18.38	18.75	18.91	18.82	18.48	17.89	17.06	15.99	14.71	13.24	11.60	9.82	7.93	5.94
3.4	16.69	17.67	18.66	19.63	20.55	21.37	22.06	22.58	22.88	22.94	22.75	22.29	21.56	20.57	19.34	17.89	16.25	14.44	12.49	10.43	8.29
3.6	20.89	21.92	22.94	23.92	24.83	25.63	26.28	26.73	26.95	26.92	26.60	26.01	25.13	23.98	22.58	20.96	19.14	17.15	15.03	12.81	10.51
3.8	25.04	26.11	27.15	28.14	29.03	29.79	30.38	30.76	30.89	30.75	30.32	29.58	28.55	27.23	25.66	23.86	21.86	19.70	17.41	15.03	12.57
4	29.07	30.16	31.21	32.19	33.06	33.77	34.30	34.60	34.63	34.37	33.81	32.93	31.74	30.26	28.52	26.54	24.37	22.04	19.59	17.05	14.45
4.2	32.87	33.99	35.04	36.00	36.82	37.48	37.94	38.15	38.08	37.70	37.00	35.98	34.64	33.00	31.09	28.95	26.62	24.13	21.53	18.85	16.12
4.4	36.39	37.51	38.55	39.47	40.25	40.84	41.22	41.33	41.15	40.66	39.83	38.67	37.18	35.39	33.33	31.04	28.56	25.93	23.20	20.39	17.54
4.6	39.55	40.66	41.67	42.55	43.26	43.78	44.06	44.07	43.78	43.17	42.22	40.93	39.30	37.38	35.18	32.76	30.15	27.40	24.56	21.65	18.70
4.8	42.29	43.37	44.34	45.15	45.79	46.22	46.40	46.31	45.91	45.19	44.11	42.70	40.96	38.92	36.61	34.08	31.37	28.53	25.59	22.60	19.59
5	44.56	45.60	46.51	47.25	47.80	48.13	48.21	48.01	47.50	46.66	45.48	43.96	42.12	39.98	37.59	34.97	32.19	29.28	26.28	23.24	20.18
5.2	46.35	47.33	48.15	48.80	49.25	49.48	49.45	49.14	48.52	47.58	46.30	44.69	42.77	40.56	38.10	35.43	32.60	29.65	26.63	23.56	20.48
5.4	47.63	48.53	49.27	49.82	50.16	50.27	50.13	49.70	48.98	47.94	46.57	44.89	42.91	40.65	38.15	35.46	32.62	29.66	26.63	23.57	20.49
5.6	48.42	49.23	49.86	50.30	50.52	50.52	50.26	49.72	48.89	47.76	46.32	44.58	42.56	40.28	37.77	35.09	32.25	29.32	26.31	23.27	20.23
5.8	48.74	49.44	49.95	50.27	50.38	50.25	49.88	49.23	48.31	47.10	45.60	43.82	41.77	39.49	37.00	34.33	31.54	28.65	25.69	22.70	19.71
6	48.61	49.19	49.59	49.79	49.77	49.52	49.03	48.29	47.28	46.00	44.45	42.64	40.59	38.32	35.87	33.25	30.51	27.68	24.79	21.88	18.95
6.2	48.07	48.54	48.81	48.89	48.75	48.39	47.79	46.95	45.86	44.53	42.94	41.12	39.08	36.84	34.43	31.88	29.21	26.47	23.66	20.83	17.99
6.4	47.18	47.52	47.68	47.63	47.38	46.91	46.21	45.29	44.13	42.75	41.14	39.32	37.30	35.11	32.75	30.28	27.69	25.04	22.33	19.60	16.85
6.6	45.98	46.20	46.24	46.08	45.71	45.14	44.36	43.36	42.15	40.73	39.11	37.30	35.31	33.17	30.88	28.49	26.00	23.44	20.84	18.21	15.57
6.8	44.51	44.62	44.55	44.28	43.82	43.15	42.29	41.23	39.98	38.54	36.91	35.12	33.17	31.08	28.87	26.56	24.17	21.72	19.22	16.70	14.16
7	42.84	42.84	42.66	42.30	41.74	41.00	40.08	38.97	37.68	36.22	34.60	32.83	30.93	28.90	26.77	24.55	22.25	19.90	17.51	15.10	12.67

Table 42 - Error graphs for monounsaturated FAME content derived from algal cells: (1) percentage and (2) mass produced (Nitrates – $NaNO_3$, phosphates – K_2HPO_4:KH_2PO_4, iron – $FeSO_4.7H_2O$, time – days after inoculation) with key to error (red high error, blue low error) (Figure 3.10, Figure 3.11)



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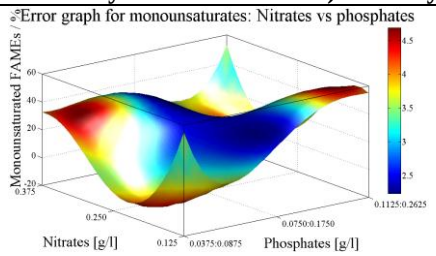

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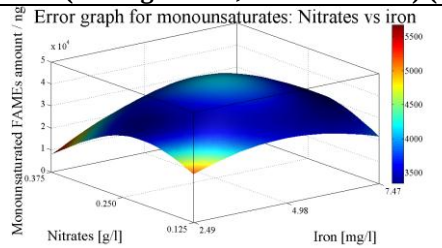

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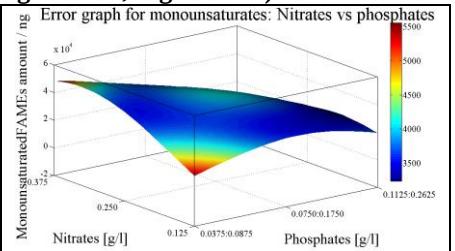

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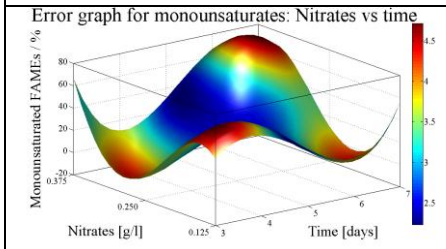

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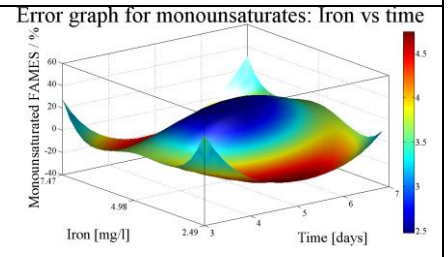

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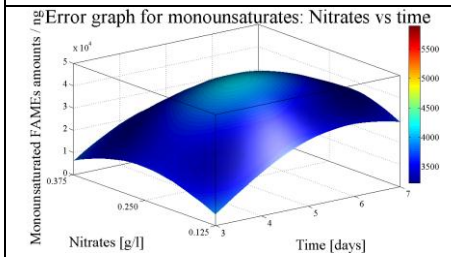

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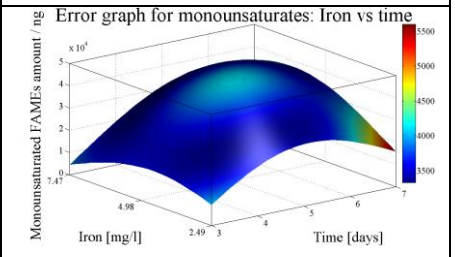

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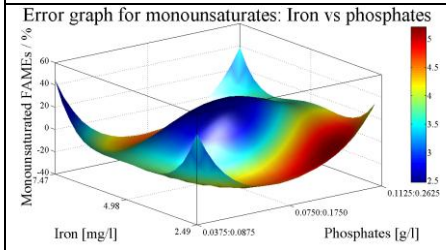

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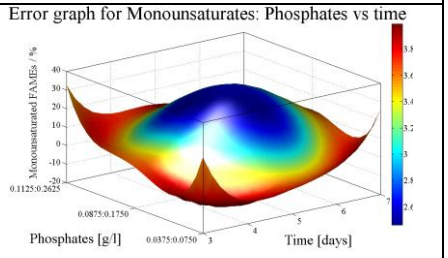

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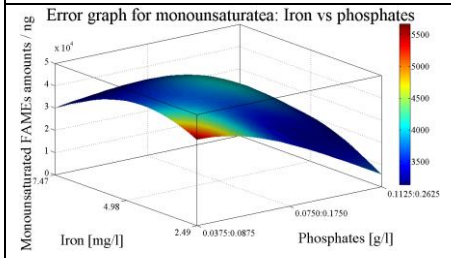
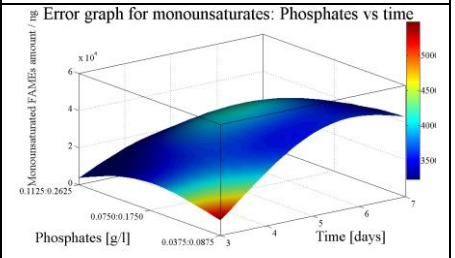


2e



2f

Table 43 - Radial basis functions determined by *MATLAB*® for polyunsaturates nitrates vs iron; where nitrates amount 5 means 0.125 g/l, 10 = 0.250 g/l and 15 = 0.375g/l; where iron amount 5 means 2.49 mg/l, 10 = 4.98 mg/l and 15 = 7.47 mg/l (Figure 3-12a)

Polyunsaturates / %	**Nitrates**																				
Iron	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
5	35.21	34.12	33.03	32.01	31.10	30.36	29.84	29.60	29.68	30.10	30.89	32.03	33.51	35.29	37.32	39.52	41.82	44.14	46.40	48.53	50.49
5.5	34.31	33.23	32.16	31.15	30.24	29.50	28.97	28.70	28.74	29.11	29.83	30.89	32.28	33.95	35.86	37.94	40.13	42.33	44.49	46.53	48.41
6	33.34	32.29	31.26	30.27	29.39	28.66	28.13	27.85	27.85	28.17	28.81	29.78	31.05	32.59	34.37	36.30	38.34	40.40	42.43	44.36	46.14
6.5	32.33	31.33	30.34	29.40	28.55	27.85	27.33	27.04	27.02	27.28	27.84	28.70	29.85	31.25	32.86	34.63	36.49	38.39	40.27	42.06	43.73
7	31.29	30.34	29.41	28.53	27.74	27.07	26.58	26.29	26.25	26.46	26.94	27.69	28.70	29.94	31.38	32.96	34.63	36.35	38.06	39.71	41.25
7.5	30.24	29.35	28.49	27.68	26.95	26.34	25.88	25.61	25.55	25.71	26.12	26.76	27.63	28.70	29.95	31.34	32.82	34.34	35.87	37.36	38.78
8	29.19	28.38	27.59	26.86	26.21	25.66	25.25	25.00	24.93	25.05	25.39	25.92	26.65	27.57	28.63	29.82	31.10	32.43	33.77	35.10	36.38
8.5	28.16	27.43	26.73	26.09	25.52	25.04	24.68	24.46	24.39	24.49	24.76	25.20	25.80	26.56	27.45	28.44	29.53	30.66	31.83	32.99	34.14
9	27.18	26.53	25.92	25.37	24.88	24.49	24.19	24.01	23.95	24.03	24.25	24.60	25.09	25.70	26.43	27.25	28.15	29.10	30.09	31.11	32.12
9.5	26.25	25.69	25.17	24.71	24.32	24.01	23.78	23.64	23.61	23.68	23.86	24.15	24.54	25.02	25.60	26.26	26.99	27.78	28.62	29.48	30.38
10	25.39	24.91	24.49	24.13	23.83	23.60	23.45	23.37	23.37	23.44	23.60	23.84	24.15	24.53	24.99	25.52	26.10	26.74	27.43	28.17	28.95
10.5	24.62	24.22	23.89	23.63	23.42	23.29	23.21	23.19	23.23	23.32	23.47	23.68	23.93	24.24	24.60	25.01	25.48	26.00	26.56	27.19	27.86
11	23.93	23.62	23.38	23.21	23.10	23.05	23.06	23.11	23.19	23.32	23.48	23.67	23.89	24.15	24.43	24.76	25.13	25.55	26.02	26.54	27.11
11.5	23.35	23.12	22.97	22.89	22.87	22.91	23.00	23.12	23.26	23.43	23.61	23.81	24.02	24.24	24.48	24.75	25.05	25.39	25.78	26.22	26.71
12	22.87	22.72	22.65	22.66	22.73	22.86	23.03	23.22	23.43	23.65	23.87	24.08	24.30	24.51	24.73	24.97	25.22	25.51	25.83	26.21	26.64
12.5	22.52	22.43	22.44	22.53	22.69	22.90	23.15	23.42	23.70	23.97	24.24	24.49	24.72	24.95	25.17	25.39	25.62	25.87	26.15	26.47	26.85
13	22.28	22.26	22.34	22.51	22.75	23.04	23.36	23.71	24.05	24.39	24.71	25.00	25.28	25.52	25.76	25.98	26.20	26.44	26.69	26.98	27.31
13.5	22.17	22.21	22.36	22.59	22.90	23.27	23.67	24.08	24.50	24.90	25.27	25.62	25.93	26.22	26.48	26.72	26.95	27.18	27.42	27.69	27.99
14	22.20	22.29	22.49	22.79	23.16	23.59	24.06	24.54	25.02	25.48	25.92	26.32	26.68	27.00	27.30	27.57	27.81	28.06	28.30	28.55	28.83
14.5	22.36	22.50	22.75	23.10	23.53	24.01	24.54	25.08	25.61	26.13	26.63	27.08	27.49	27.86	28.20	28.50	28.77	29.03	29.27	29.52	29.78
15	22.65	22.83	23.12	23.52	23.99	24.53	25.10	25.69	26.28	26.85	27.39	27.89	28.35	28.77	29.14	29.48	29.78	30.06	30.32	30.57	30.82

Table 44 - Radial basis functions determined by *MATLAB*® for polyunsaturates nitrates vs phosphates; where nitrates amount 5 means 0.125 g/l, 10 = 0.250 g/l and 15 = 0.375g/l; where phosphates of K_2HPO_4:KH_2PO_4 of 0.0375:0.0875 g/l, 10 = 0.0750:0.1750 g/l and 15 = 0.1125:0.2625 g/l (Figure 3-12b)

Polyunsaturates / %	**Nitrates**																				
Phosphates	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
5	27.27	26.82	26.47	26.21	26.05	25.99	26.04	26.20	26.47	26.84	27.31	27.88	28.55	29.30	30.12	31.01	31.95	32.94	33.95	34.99	36.03
5.5	27.05	26.59	26.22	25.94	25.76	25.68	25.70	25.83	26.08	26.43	26.88	27.43	28.08	28.82	29.63	30.52	31.45	32.43	33.44	34.47	35.51
6	26.88	26.41	26.02	25.72	25.51	25.41	25.41	25.51	25.73	26.05	26.48	27.02	27.65	28.36	29.16	30.03	30.95	31.92	32.92	33.94	34.97
6.5	26.74	26.26	25.85	25.53	25.31	25.18	25.15	25.23	25.41	25.71	26.11	26.62	27.22	27.92	28.70	29.54	30.45	31.40	32.38	33.39	34.40
7	26.62	26.13	25.71	25.38	25.13	24.97	24.92	24.97	25.12	25.39	25.76	26.23	26.81	27.48	28.23	29.05	29.93	30.86	31.82	32.80	33.79
7.5	26.51	26.01	25.58	25.23	24.96	24.78	24.70	24.72	24.84	25.08	25.41	25.86	26.40	27.03	27.75	28.54	29.39	30.28	31.21	32.17	33.14
8	26.37	25.87	25.44	25.07	24.79	24.59	24.48	24.48	24.57	24.77	25.07	25.48	25.98	26.57	27.25	28.00	28.81	29.67	30.57	31.49	32.43
8.5	26.21	25.71	25.27	24.89	24.60	24.38	24.26	24.23	24.29	24.46	24.72	25.09	25.55	26.10	26.73	27.43	28.20	29.01	29.87	30.75	31.66
9	25.99	25.50	25.06	24.68	24.38	24.16	24.01	23.96	24.00	24.14	24.37	24.69	25.10	25.60	26.18	26.83	27.54	28.31	29.11	29.95	30.82
9.5	25.72	25.23	24.80	24.43	24.13	23.90	23.75	23.68	23.69	23.80	23.99	24.27	24.64	25.08	25.60	26.19	26.84	27.55	28.30	29.09	29.92
10	25.39	24.91	24.49	24.13	23.83	23.60	23.45	23.37	23.37	23.44	23.60	23.84	24.15	24.53	24.99	25.52	26.10	26.74	27.43	28.17	28.95
10.5	25.00	24.53	24.13	23.78	23.50	23.28	23.12	23.04	23.02	23.07	23.20	23.39	23.64	23.97	24.36	24.81	25.32	25.89	26.52	27.20	27.92
11	24.55	24.10	23.71	23.39	23.12	22.92	22.77	22.69	22.66	22.69	22.78	22.92	23.13	23.39	23.71	24.08	24.52	25.01	25.57	26.18	26.86
11.5	24.05	23.62	23.26	22.96	22.72	22.53	22.40	22.32	22.29	22.30	22.36	22.46	22.61	22.80	23.04	23.34	23.70	24.12	24.60	25.15	25.77
12	23.52	23.12	22.78	22.51	22.29	22.13	22.02	21.95	21.91	21.91	21.94	22.00	22.09	22.22	22.39	22.61	22.88	23.22	23.63	24.11	24.67
12.5	22.99	22.61	22.30	22.05	21.87	21.73	21.64	21.58	21.54	21.53	21.53	21.55	21.59	21.65	21.75	21.89	22.09	22.35	22.68	23.10	23.60
13	22.46	22.10	21.82	21.61	21.45	21.35	21.27	21.23	21.19	21.17	21.15	21.13	21.12	21.12	21.15	21.22	21.33	21.52	21.78	22.13	22.59
13.5	21.98	21.64	21.38	21.20	21.07	20.99	20.94	20.91	20.88	20.84	20.80	20.75	20.69	20.64	20.60	20.60	20.65	20.76	20.95	21.25	21.65
14	21.55	21.23	21.00	20.84	20.74	20.68	20.65	20.63	20.60	20.56	20.50	20.42	20.32	20.22	20.13	20.06	20.04	20.09	20.22	20.46	20.82
14.5	21.20	20.90	20.69	20.56	20.48	20.44	20.42	20.41	20.38	20.33	20.26	20.15	20.02	19.87	19.73	19.61	19.54	19.53	19.61	19.80	20.12
15	20.96	20.67	20.47	20.36	20.29	20.27	20.26	20.26	20.23	20.17	20.08	19.95	19.79	19.61	19.43	19.27	19.15	19.10	19.14	19.29	19.56

Table 45 - Radial basis functions determined by *MATLAB*® for polyunsaturates nitrates vs time; where nitrates amount 5 means 0.125 g/l, 10 = 0.250 g/l and 15 = 0.375g/l (Figure 3-12c)

Polyunsaturates / %	**Nitrates**																				
Time / days	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
3	33.55	33.22	32.90	32.57	32.23	31.85	31.44	30.97	30.46	29.89	29.26	28.60	27.90	27.19	26.49	25.83	25.25	24.77	24.43	24.25	24.26
3.2	32.47	32.14	31.83	31.51	31.19	30.84	30.47	30.06	29.61	29.11	28.57	27.99	27.39	26.78	26.19	25.64	25.16	24.78	24.53	24.44	24.51
3.4	31.41	31.08	30.77	30.47	30.16	29.85	29.52	29.16	28.78	28.36	27.90	27.43	26.93	26.44	25.97	25.54	25.18	24.91	24.77	24.76	24.92
3.6	30.39	30.05	29.74	29.44	29.16	28.87	28.58	28.28	27.96	27.63	27.27	26.90	26.53	26.16	25.82	25.52	25.29	25.15	25.12	25.21	25.45
3.8	29.42	29.07	28.75	28.46	28.18	27.93	27.68	27.43	27.18	26.92	26.66	26.41	26.15	25.92	25.71	25.56	25.46	25.45	25.54	25.74	26.06
4	28.51	28.14	27.82	27.52	27.26	27.03	26.81	26.61	26.42	26.25	26.09	25.94	25.81	25.70	25.64	25.62	25.66	25.78	25.99	26.29	26.70
4.2	27.68	27.29	26.96	26.66	26.40	26.18	25.99	25.84	25.71	25.61	25.54	25.49	25.48	25.50	25.57	25.69	25.86	26.11	26.43	26.84	27.34
4.4	26.94	26.54	26.18	25.88	25.62	25.41	25.24	25.12	25.04	25.01	25.01	25.06	25.15	25.29	25.48	25.73	26.03	26.40	26.83	27.34	27.91
4.6	26.31	25.88	25.51	25.19	24.92	24.71	24.56	24.47	24.43	24.44	24.51	24.64	24.83	25.07	25.37	25.72	26.14	26.61	27.15	27.74	28.39
4.8	25.79	25.34	24.94	24.60	24.32	24.11	23.96	23.88	23.87	23.92	24.04	24.23	24.49	24.82	25.21	25.66	26.17	26.73	27.35	28.03	28.74
5	25.39	24.91	24.49	24.13	23.83	23.60	23.45	23.37	23.37	23.44	23.60	23.84	24.15	24.53	24.99	25.52	26.10	26.74	27.43	28.17	28.95
5.2	25.12	24.61	24.16	23.77	23.45	23.20	23.03	22.94	22.93	23.02	23.19	23.45	23.79	24.22	24.72	25.30	25.94	26.63	27.38	28.17	28.99
5.4	24.96	24.43	23.95	23.53	23.18	22.90	22.70	22.59	22.57	22.64	22.81	23.07	23.43	23.87	24.40	25.00	25.67	26.40	27.19	28.01	28.87
5.6	24.93	24.37	23.86	23.40	23.01	22.70	22.47	22.32	22.27	22.32	22.47	22.72	23.06	23.50	24.03	24.64	25.32	26.07	26.87	27.72	28.61
5.8	25.00	24.41	23.87	23.38	22.95	22.60	22.32	22.14	22.05	22.06	22.17	22.38	22.70	23.12	23.63	24.23	24.90	25.65	26.45	27.31	28.21
6	25.17	24.56	23.98	23.45	22.98	22.58	22.26	22.03	21.89	21.85	21.91	22.09	22.36	22.74	23.21	23.78	24.43	25.16	25.96	26.81	27.72
6.2	25.42	24.78	24.18	23.61	23.10	22.65	22.28	21.99	21.80	21.70	21.71	21.83	22.05	22.38	22.80	23.33	23.94	24.64	25.41	26.26	27.17
6.4	25.74	25.08	24.44	23.84	23.29	22.79	22.37	22.02	21.77	21.62	21.56	21.62	21.78	22.04	22.42	22.89	23.45	24.11	24.86	25.68	26.58
6.6	26.11	25.43	24.76	24.13	23.53	22.99	22.52	22.12	21.81	21.59	21.48	21.47	21.56	21.76	22.07	22.49	23.00	23.62	24.33	25.12	26.00
6.8	26.52	25.81	25.13	24.46	23.83	23.25	22.73	22.28	21.91	21.63	21.46	21.38	21.41	21.55	21.79	22.15	22.61	23.17	23.84	24.61	25.47
7	26.94	26.23	25.52	24.83	24.17	23.55	22.99	22.49	22.07	21.74	21.50	21.36	21.33	21.41	21.59	21.89	22.30	22.81	23.44	24.18	25.01

Table 46 - Radial basis functions determined by *MATLAB*® for polyunsaturates iron vs time; where iron amount 5 means 2.49 mg/l, 10 = 4.98 mg/l and 15 = 7.47 mg/l (Figure 3-12d)

Polyunsaturates / %	**Iron**																				
Time / days	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
3	31.38	30.92	30.55	30.26	30.04	29.87	29.74	29.62	29.51	29.40	29.26	29.12	28.96	28.79	28.63	28.50	28.40	28.36	28.39	28.51	28.73
3.2	31.10	30.58	30.16	29.81	29.54	29.32	29.14	28.99	28.85	28.71	28.57	28.42	28.27	28.13	27.99	27.89	27.84	27.84	27.92	28.09	28.35
3.4	30.91	30.34	29.85	29.45	29.11	28.83	28.60	28.40	28.22	28.06	27.90	27.76	27.62	27.50	27.40	27.34	27.34	27.39	27.53	27.75	28.06
3.6	30.80	30.16	29.61	29.14	28.74	28.39	28.10	27.85	27.63	27.44	27.27	27.12	27.00	26.91	26.85	26.85	26.89	27.01	27.20	27.48	27.85
3.8	30.75	30.06	29.44	28.89	28.41	28.00	27.64	27.33	27.07	26.85	26.66	26.52	26.42	26.36	26.35	26.39	26.50	26.68	26.94	27.28	27.71
4	30.75	29.99	29.30	28.68	28.12	27.63	27.21	26.84	26.54	26.28	26.09	25.95	25.86	25.83	25.87	25.97	26.15	26.40	26.72	27.13	27.62
4.2	30.78	29.96	29.20	28.50	27.87	27.30	26.81	26.38	26.03	25.75	25.54	25.40	25.33	25.34	25.43	25.59	25.83	26.14	26.54	27.01	27.56
4.4	30.83	29.94	29.11	28.33	27.62	26.99	26.43	25.95	25.55	25.24	25.01	24.88	24.83	24.87	25.01	25.22	25.53	25.91	26.38	26.92	27.52
4.6	30.87	29.92	29.02	28.17	27.39	26.69	26.07	25.53	25.09	24.75	24.51	24.38	24.35	24.43	24.61	24.88	25.25	25.70	26.22	26.82	27.49
4.8	30.89	29.88	28.92	28.01	27.17	26.40	25.72	25.14	24.66	24.29	24.04	23.91	23.90	24.01	24.23	24.55	24.97	25.48	26.07	26.73	27.45
5	30.89	29.83	28.81	27.84	26.94	26.12	25.39	24.76	24.25	23.86	23.60	23.47	23.48	23.61	23.87	24.24	24.71	25.27	25.92	26.63	27.39
5.2	30.85	29.75	28.68	27.66	26.71	25.84	25.06	24.40	23.86	23.46	23.19	23.07	23.08	23.24	23.53	23.94	24.45	25.06	25.75	26.51	27.32
5.4	30.79	29.64	28.53	27.47	26.48	25.57	24.76	24.07	23.50	23.08	22.81	22.69	22.72	22.89	23.21	23.65	24.20	24.85	25.58	26.38	27.22
5.6	30.69	29.51	28.37	27.28	26.25	25.31	24.47	23.76	23.18	22.75	22.47	22.35	22.39	22.58	22.91	23.38	23.96	24.64	25.40	26.23	27.11
5.8	30.57	29.37	28.20	27.08	26.03	25.07	24.21	23.48	22.89	22.45	22.17	22.05	22.10	22.30	22.65	23.13	23.74	24.44	25.23	26.08	26.98
6	30.43	29.21	28.02	26.89	25.82	24.84	23.98	23.24	22.64	22.19	21.91	21.80	21.85	22.06	22.42	22.92	23.53	24.25	25.06	25.93	26.85
6.2	30.28	29.05	27.85	26.71	25.63	24.65	23.78	23.03	22.44	21.99	21.71	21.60	21.65	21.87	22.23	22.74	23.36	24.09	24.90	25.79	26.72
6.4	30.13	28.90	27.70	26.55	25.48	24.49	23.62	22.88	22.28	21.84	21.56	21.45	21.51	21.73	22.09	22.60	23.22	23.96	24.77	25.66	26.60
6.6	30.00	28.76	27.57	26.42	25.36	24.38	23.52	22.78	22.19	21.75	21.48	21.37	21.42	21.64	22.00	22.51	23.13	23.86	24.68	25.56	26.51
6.8	29.89	28.66	27.47	26.34	25.28	24.32	23.46	22.74	22.16	21.73	21.46	21.35	21.40	21.61	21.97	22.47	23.09	23.81	24.62	25.50	26.44
7	29.81	28.59	27.41	26.30	25.26	24.31	23.47	22.76	22.19	21.77	21.50	21.39	21.45	21.65	22.01	22.49	23.10	23.81	24.61	25.48	26.41

Table 47 - Radial basis functions determined by *MATLAB*® for polyunsaturates iron vs phosphates; where iron amount 5 means 2.49 mg/l, 10 = 4.98 mg/l and 15 = 7.47 mg/l; where phosphate amount 5 means a ratio of K_2HPO_4:KH_2PO_4 of 0.0375:0.0875 g/l, 10 = 0.0750:0.1750 g/l and 15 = 0.1125:0.2625 g/l (Figure 3-12e,)

Polyunsaturates / %	**Iron**																				
Phosphates	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
5	30.08	29.39	28.82	28.37	28.03	27.78	27.60	27.47	27.39	27.34	27.31	27.29	27.29	27.31	27.34	27.41	27.50	27.64	27.83	28.06	28.36
5.5	30.39	29.64	29.01	28.49	28.07	27.74	27.47	27.27	27.10	26.98	26.88	26.81	26.76	26.73	26.74	26.78	26.87	27.01	27.20	27.45	27.77
6	30.77	29.97	29.28	28.68	28.18	27.75	27.40	27.10	26.86	26.65	26.48	26.35	26.25	26.19	26.18	26.21	26.29	26.44	26.64	26.92	27.26
6.5	31.20	30.34	29.58	28.91	28.31	27.80	27.35	26.96	26.63	26.35	26.11	25.92	25.78	25.70	25.66	25.69	25.78	25.94	26.16	26.46	26.84
7	31.60	30.70	29.87	29.12	28.44	27.84	27.30	26.82	26.41	26.05	25.76	25.52	25.35	25.24	25.20	25.23	25.34	25.52	25.77	26.11	26.52
7.5	31.94	30.99	30.10	29.28	28.52	27.83	27.21	26.66	26.17	25.76	25.41	25.14	24.95	24.83	24.80	24.85	24.98	25.19	25.48	25.86	26.32
8	32.15	31.16	30.22	29.34	28.51	27.75	27.06	26.45	25.91	25.45	25.07	24.78	24.59	24.48	24.46	24.54	24.71	24.97	25.31	25.74	26.24
8.5	32.19	31.17	30.18	29.25	28.37	27.56	26.82	26.16	25.59	25.11	24.72	24.44	24.26	24.18	24.20	24.32	24.54	24.86	25.26	25.75	26.31
9	32.01	30.96	29.95	28.99	28.08	27.24	26.47	25.80	25.21	24.74	24.37	24.11	23.96	23.93	24.01	24.20	24.49	24.87	25.34	25.90	26.52
9.5	31.58	30.52	29.50	28.52	27.61	26.76	26.00	25.33	24.77	24.32	23.99	23.79	23.70	23.74	23.90	24.17	24.54	25.01	25.56	26.19	26.88
10	30.89	29.83	28.81	27.84	26.94	26.12	25.39	24.76	24.25	23.86	23.60	23.47	23.48	23.61	23.87	24.24	24.71	25.27	25.92	26.63	27.39
10.5	29.93	28.89	27.89	26.95	26.09	25.31	24.64	24.08	23.65	23.36	23.20	23.17	23.29	23.54	23.91	24.40	24.98	25.66	26.40	27.20	28.04
11	28.73	27.70	26.74	25.85	25.06	24.36	23.77	23.32	22.99	22.81	22.78	22.89	23.14	23.52	24.03	24.65	25.36	26.15	27.00	27.89	28.81
11.5	27.30	26.32	25.41	24.59	23.87	23.27	22.80	22.47	22.28	22.24	22.36	22.62	23.02	23.56	24.22	24.98	25.82	26.74	27.70	28.69	29.69
12	25.70	24.76	23.92	23.18	22.57	22.09	21.76	21.57	21.54	21.66	21.94	22.37	22.94	23.64	24.46	25.37	26.36	27.40	28.48	29.57	30.65
12.5	23.98	23.10	22.33	21.69	21.20	20.86	20.67	20.64	20.78	21.07	21.53	22.14	22.89	23.76	24.75	25.82	26.95	28.13	29.32	30.50	31.66
13	22.21	21.38	20.70	20.17	19.80	19.60	19.57	19.71	20.03	20.51	21.15	21.94	22.87	23.92	25.07	26.29	27.57	28.88	30.18	31.46	32.70
13.5	20.44	19.67	19.08	18.66	18.43	18.38	18.51	18.82	19.31	19.98	20.80	21.77	22.88	24.10	25.41	26.79	28.21	29.64	31.05	32.42	33.74
14	18.74	18.04	17.54	17.24	17.13	17.23	17.52	18.00	18.66	19.50	20.50	21.65	22.92	24.30	25.77	27.29	28.83	30.38	31.89	33.35	34.73
14.5	17.16	16.54	16.13	15.93	15.95	16.19	16.63	17.27	18.09	19.09	20.26	21.56	22.99	24.52	26.12	27.77	29.44	31.08	32.69	34.22	35.66
15	15.77	15.22	14.90	14.80	14.94	15.30	15.87	16.65	17.63	18.77	20.08	21.53	23.09	24.75	26.47	28.23	30.00	31.73	33.41	35.01	36.51

Table 48 - Radial basis functions determined by *MATLAB*® for polyunsaturates phosphates vs time; where phosphate amount 5 means a ratio of K_2HPO_4:KH_2PO_4 of 0.0375:0.0875 g/l, 10 = 0.0750:0.1750 g/l and 15 = 0.1125:0.2625 g/l (Figure 3-12f)

Polyunsaturates / %	**Phosphates**																				
Time / days	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
3	37.89	36.78	35.62	34.45	33.31	32.24	31.29	30.48	29.86	29.45	29.26	29.31	29.57	30.04	30.68	31.46	32.33	33.24	34.16	35.03	35.84
3.2	36.90	35.83	34.72	33.61	32.52	31.50	30.58	29.80	29.19	28.77	28.57	28.58	28.79	29.19	29.76	30.45	31.23	32.05	32.87	33.66	34.39
3.4	35.84	34.83	33.79	32.74	31.72	30.75	29.88	29.14	28.54	28.13	27.90	27.87	28.03	28.35	28.82	29.41	30.08	30.79	31.50	32.20	32.83
3.6	34.72	33.78	32.81	31.84	30.90	30.01	29.20	28.49	27.93	27.52	27.27	27.19	27.28	27.51	27.88	28.35	28.89	29.48	30.07	30.65	31.19
3.8	33.57	32.70	31.82	30.94	30.08	29.27	28.53	27.88	27.34	26.93	26.66	26.54	26.55	26.69	26.94	27.28	27.69	28.13	28.60	29.05	29.49
4	32.41	31.62	30.83	30.04	29.27	28.55	27.88	27.28	26.78	26.38	26.09	25.91	25.84	25.88	26.01	26.22	26.48	26.78	27.11	27.44	27.76
4.2	31.27	30.56	29.86	29.16	28.49	27.85	27.25	26.71	26.24	25.85	25.54	25.31	25.16	25.10	25.11	25.18	25.29	25.45	25.64	25.84	26.06
4.4	30.17	29.54	28.92	28.31	27.73	27.18	26.65	26.17	25.73	25.34	25.01	24.74	24.52	24.35	24.24	24.17	24.15	24.17	24.22	24.30	24.40
4.6	29.13	28.57	28.03	27.52	27.02	26.54	26.09	25.66	25.25	24.87	24.51	24.19	23.90	23.64	23.41	23.22	23.07	22.95	22.87	22.83	22.84
4.8	28.17	27.68	27.22	26.78	26.36	25.95	25.56	25.17	24.79	24.42	24.04	23.68	23.32	22.97	22.64	22.34	22.06	21.82	21.63	21.48	21.39
5	27.31	26.88	26.48	26.11	25.76	25.41	25.07	24.72	24.37	23.99	23.60	23.20	22.78	22.36	21.94	21.53	21.15	20.80	20.50	20.26	20.08
5.2	26.56	26.18	25.84	25.52	25.22	24.93	24.63	24.31	23.97	23.60	23.19	22.75	22.28	21.80	21.30	20.81	20.33	19.90	19.51	19.18	18.93
5.4	25.92	25.58	25.28	25.01	24.76	24.50	24.23	23.94	23.61	23.23	22.81	22.34	21.83	21.30	20.74	20.18	19.63	19.12	18.66	18.26	17.95
5.6	25.40	25.09	24.83	24.59	24.36	24.13	23.89	23.61	23.28	22.90	22.47	21.98	21.44	20.86	20.25	19.64	19.04	18.47	17.95	17.51	17.15
5.8	25.00	24.71	24.47	24.25	24.04	23.83	23.59	23.32	23.00	22.61	22.17	21.66	21.10	20.49	19.85	19.20	18.56	17.95	17.40	16.91	16.52
6	24.71	24.44	24.20	23.99	23.79	23.59	23.36	23.08	22.76	22.37	21.91	21.40	20.82	20.19	19.53	18.86	18.20	17.57	16.98	16.48	16.06
6.2	24.53	24.26	24.03	23.82	23.62	23.41	23.18	22.90	22.57	22.17	21.71	21.19	20.60	19.97	19.30	18.62	17.95	17.30	16.71	16.19	15.76
6.4	24.46	24.18	23.94	23.73	23.52	23.30	23.06	22.77	22.43	22.03	21.56	21.04	20.45	19.82	19.15	18.47	17.80	17.16	16.56	16.04	15.61
6.6	24.47	24.19	23.94	23.71	23.49	23.26	23.00	22.70	22.35	21.95	21.48	20.95	20.37	19.74	19.09	18.42	17.76	17.13	16.54	16.02	15.59
6.8	24.58	24.28	24.02	23.77	23.53	23.28	23.01	22.70	22.34	21.92	21.46	20.93	20.36	19.75	19.11	18.46	17.81	17.20	16.63	16.13	15.71
7	24.76	24.45	24.17	23.90	23.64	23.37	23.08	22.75	22.39	21.97	21.50	20.98	20.42	19.83	19.21	18.58	17.96	17.37	16.83	16.34	15.94

Table 49 - Radial basis functions determined by *MATLAB*® for polyunsaturates mass nitrates vs iron; where nitrates amount 5 means 0.125 g/l, 10 = 0.250 g/l and 15 = 0.375g/l; where iron amount 5 means 2.49 mg/l, 10 = 4.98 mg/l and 15 = 7.47 mg/l (Figure 3-13a)

Polyunsaturates / mg	**Nitrates**																				
Iron	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
5	35.90	36.30	36.12	35.54	34.71	33.72	32.65	31.49	30.26	28.92	27.47	25.89	24.17	22.30	20.31	18.21	16.05	13.92	11.92	10.21	8.97
5.5	35.45	36.01	36.05	35.71	35.13	34.40	33.58	32.67	31.65	30.51	29.23	27.77	26.14	24.34	22.39	20.31	18.16	16.02	14.01	12.28	10.98
6	34.41	35.18	35.50	35.49	35.26	34.88	34.40	33.80	33.07	32.18	31.10	29.81	28.30	26.59	24.70	22.68	20.57	18.48	16.50	14.78	13.44
6.5	32.99	33.97	34.62	34.99	35.17	35.21	35.12	34.90	34.51	33.91	33.07	31.97	30.60	29.00	27.19	25.23	23.19	21.16	19.24	17.54	16.16
7	31.38	32.58	33.55	34.32	34.94	35.42	35.78	35.98	35.98	35.71	35.14	34.23	33.00	31.49	29.75	27.86	25.88	23.91	22.04	20.35	18.91
7.5	29.73	31.15	32.43	33.58	34.63	35.58	36.40	37.05	37.45	37.54	37.24	36.53	35.43	33.99	32.30	30.44	28.50	26.57	24.72	23.02	21.53
8	28.14	29.76	31.32	32.83	34.29	35.68	36.97	38.07	38.90	39.35	39.32	38.79	37.78	36.38	34.69	32.84	30.91	28.99	27.14	25.43	23.88
8.5	26.68	28.47	30.29	32.11	33.94	35.74	37.45	38.99	40.24	41.04	41.28	40.89	39.93	38.52	36.81	34.93	32.98	31.05	29.19	27.44	25.83
9	25.39	27.33	29.34	31.43	33.56	35.70	37.79	39.71	41.34	42.46	42.93	42.65	41.70	40.25	38.49	36.56	34.59	32.63	30.75	28.97	27.31
9.5	24.30	26.34	28.51	30.78	33.13	35.53	37.89	40.11	42.03	43.41	44.05	43.84	42.89	41.40	39.59	37.63	35.63	33.65	31.74	29.94	28.25
10	23.42	25.52	27.78	30.16	32.64	35.18	37.70	40.08	42.15	43.67	44.41	44.26	43.33	41.83	40.02	38.04	36.03	34.04	32.13	30.31	28.60
10.5	22.75	24.88	27.16	29.57	32.08	34.65	37.18	39.57	41.64	43.16	43.91	43.80	42.93	41.49	39.72	37.77	35.77	33.79	31.88	30.06	28.36
11	22.28	24.39	26.64	29.01	31.46	33.94	36.37	38.63	40.56	41.96	42.65	42.57	41.77	40.42	38.73	36.84	34.88	32.91	31.01	29.20	27.52
11.5	22.01	24.06	26.22	28.48	30.79	33.10	35.33	37.37	39.07	40.29	40.87	40.77	40.03	38.77	37.16	35.34	33.40	31.46	29.57	27.78	26.13
12	21.91	23.87	25.91	28.00	30.11	32.19	34.16	35.93	37.36	38.35	38.79	38.63	37.90	36.71	35.16	33.37	31.46	29.51	27.62	25.85	24.25
12.5	21.94	23.79	25.67	27.56	29.44	31.25	32.93	34.40	35.56	36.31	36.58	36.33	35.57	34.39	32.85	31.07	29.15	27.18	25.28	23.51	21.95
13	22.08	23.80	25.49	27.16	28.77	30.30	31.69	32.86	33.75	34.26	34.35	33.98	33.16	31.94	30.38	28.57	26.61	24.60	22.65	20.88	19.37
13.5	22.26	23.84	25.34	26.77	28.11	29.36	30.46	31.35	31.97	32.27	32.17	31.68	30.77	29.49	27.88	26.01	23.99	21.91	19.90	18.10	16.62
14	22.42	23.85	25.15	26.34	27.43	28.41	29.24	29.88	30.26	30.35	30.09	29.46	28.46	27.11	25.44	23.52	21.42	19.27	17.19	15.35	13.92
14.5	22.46	23.75	24.86	25.84	26.70	27.44	28.03	28.44	28.62	28.53	28.12	27.37	26.29	24.87	23.15	21.19	19.05	16.84	14.72	12.87	11.47
15	22.30	23.46	24.42	25.21	25.88	26.42	26.82	27.04	27.05	26.80	26.27	25.43	24.28	22.82	21.09	19.12	16.98	14.78	12.68	10.86	9.52

Table 50 - Radial basis functions determined by *MATLAB*® for polyunsaturates mass nitrates vs phosphates; where nitrates amount 5 means 0.125 g/l, 10 = 0.250 g/l and 15 = 0.375g/l; where phosphates of K_2HPO_4:KH_2PO_4 of 0.0375:0.0875 g/l, 10 = 0.0750:0.1750 g/l and 15 = 0.1125:0.2625 g/l (Figure 3-13b)

Polyunsaturates / mg	**Nitrates**																				
Phosphates	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
5	5.34	7.76	10.85	14.41	18.22	22.11	25.97	29.74	33.37	36.85	40.16	43.33	46.36	49.28	52.10	54.81	57.38	59.70	61.56	62.67	62.73
5.5	6.69	9.13	12.22	15.75	19.52	23.36	27.16	30.84	34.36	37.68	40.80	43.74	46.50	49.12	51.61	53.98	56.21	58.19	59.73	60.57	60.43
6	8.66	11.09	14.11	17.53	21.18	24.91	28.58	32.13	35.48	38.60	41.47	44.10	46.51	48.72	50.78	52.69	54.42	55.91	56.99	57.44	57.06
6.5	11.00	13.42	16.34	19.62	23.11	26.68	30.19	33.56	36.71	39.59	42.16	44.43	46.42	48.15	49.68	51.03	52.18	53.08	53.61	53.63	52.98
7	13.48	15.88	18.70	21.83	25.16	28.55	31.90	35.09	38.02	40.64	42.89	44.76	46.27	47.47	48.41	49.14	49.66	49.94	49.93	49.51	48.61
7.5	15.90	18.28	21.01	24.01	27.18	30.42	33.60	36.61	39.35	41.71	43.63	45.09	46.10	46.73	47.06	47.15	47.04	46.73	46.19	45.38	44.24
8	18.12	20.48	23.13	26.01	29.05	32.14	35.18	38.05	40.61	42.75	44.35	45.39	45.90	45.96	45.68	45.16	44.46	43.61	42.62	41.45	40.11
8.5	20.04	22.37	24.94	27.71	30.62	33.59	36.51	39.26	41.69	43.64	44.96	45.61	45.63	45.15	44.31	43.24	42.03	40.72	39.33	37.89	36.38
9	21.59	23.87	26.35	29.01	31.80	34.65	37.46	40.11	42.43	44.23	45.31	45.62	45.21	44.25	42.94	41.42	39.79	38.12	36.45	34.79	33.16
9.5	22.73	24.93	27.31	29.84	32.49	35.20	37.89	40.42	42.64	44.31	45.20	45.23	44.49	43.18	41.52	39.69	37.79	35.89	34.03	32.25	30.55
10	23.42	25.52	27.78	30.16	32.64	35.18	37.70	40.08	42.15	43.67	44.41	44.26	43.33	41.83	40.02	38.04	36.03	34.04	32.13	30.31	28.60
10.5	23.67	25.65	27.75	29.95	32.24	34.57	36.87	39.03	40.89	42.24	42.85	42.63	41.68	40.20	38.42	36.50	34.54	32.61	30.76	29.00	27.34
11	23.49	25.32	27.24	29.24	31.30	33.39	35.43	37.33	38.95	40.10	40.62	40.43	39.61	38.33	36.76	35.06	33.32	31.60	29.92	28.31	26.76
11.5	22.92	24.58	26.31	28.09	29.91	31.73	33.50	35.13	36.51	37.49	37.96	37.88	37.29	36.33	35.12	33.78	32.39	31.00	29.61	28.22	26.83
12	22.01	23.49	25.01	26.56	28.14	29.70	31.20	32.58	33.75	34.62	35.10	35.18	34.89	34.31	33.54	32.67	31.75	30.78	29.77	28.68	27.49
12.5	20.82	22.11	23.42	24.76	26.09	27.41	28.68	29.85	30.86	31.66	32.21	32.48	32.51	32.36	32.09	31.74	31.35	30.89	30.33	29.60	28.66
13	19.41	20.52	21.63	22.75	23.87	24.97	26.04	27.05	27.95	28.74	29.37	29.86	30.23	30.51	30.75	30.96	31.14	31.24	31.19	30.86	30.18
13.5	17.85	18.78	19.71	20.64	21.56	22.48	23.39	24.27	25.11	25.91	26.66	27.37	28.06	28.76	29.49	30.25	31.01	31.69	32.17	32.27	31.87
14	16.20	16.96	17.72	18.48	19.25	20.01	20.79	21.58	22.39	23.23	24.10	25.02	26.01	27.09	28.26	29.52	30.81	32.04	33.04	33.57	33.44
14.5	14.50	15.12	15.74	16.35	16.98	17.63	18.31	19.04	19.84	20.72	21.70	22.80	24.05	25.44	26.98	28.65	30.39	32.08	33.52	34.43	34.54
15	12.81	13.30	13.80	14.29	14.81	15.37	15.98	16.67	17.47	18.39	19.47	20.72	22.15	23.78	25.59	27.56	29.61	31.61	33.34	34.52	34.83

Table 51 - Radial basis functions determined by *MATLAB*® for polyunsaturates mass nitrates vs time; where nitrates amount 5 means 0.125 g/l, 10 = 0.250 g/l and 15 = 0.375g/l (Figure 3-13c)

Polyunsaturates / mg	**Nitrates**																				
Time / days	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
3	6.15	6.65	7.13	7.59	8.04	8.45	8.82	9.14	9.40	9.59	9.70	9.72	9.65	9.47	9.20	8.84	8.41	7.94	7.48	7.12	6.93
3.2	8.22	8.86	9.49	10.12	10.73	11.30	11.82	12.28	12.66	12.94	13.11	13.15	13.07	12.86	12.52	12.09	11.56	11.00	10.44	9.96	9.64
3.4	10.31	11.12	11.93	12.74	13.54	14.29	15.00	15.62	16.14	16.53	16.76	16.83	16.73	16.48	16.08	15.56	14.94	14.28	13.63	13.04	12.59
3.6	12.41	13.40	14.40	15.41	16.42	17.39	18.30	19.12	19.80	20.31	20.62	20.72	20.60	20.28	19.79	19.17	18.45	17.69	16.93	16.23	15.63
3.8	14.48	15.65	16.85	18.09	19.32	20.53	21.68	22.72	23.59	24.24	24.63	24.74	24.58	24.17	23.57	22.82	21.98	21.09	20.20	19.36	18.61
4	16.45	17.81	19.23	20.69	22.17	23.64	25.04	26.33	27.41	28.22	28.70	28.81	28.58	28.05	27.29	26.39	25.38	24.34	23.31	22.32	21.39
4.2	18.30	19.85	21.47	23.16	24.89	26.62	28.30	29.85	31.17	32.15	32.71	32.80	32.47	31.78	30.83	29.73	28.54	27.32	26.12	24.95	23.86
4.4	19.96	21.69	23.51	25.41	27.38	29.37	31.33	33.15	34.71	35.88	36.51	36.57	36.10	35.20	34.03	32.70	31.30	29.89	28.50	27.16	25.89
4.6	21.40	23.28	25.27	27.37	29.56	31.79	33.99	36.06	37.86	39.20	39.91	39.90	39.26	38.14	36.73	35.16	33.54	31.92	30.35	28.84	27.41
4.8	22.56	24.57	26.71	28.97	31.34	33.75	36.15	38.43	40.41	41.88	42.62	42.55	41.74	40.40	38.76	36.97	35.14	33.33	31.58	29.91	28.33
5	23.42	25.52	27.78	30.16	32.64	35.18	37.70	40.08	42.15	43.67	44.41	44.26	43.33	41.83	40.02	38.04	36.03	34.04	32.13	30.31	28.60
5.2	23.95	26.12	28.45	30.90	33.45	36.04	38.59	40.97	43.01	44.47	45.15	44.94	43.94	42.37	40.44	38.33	36.16	34.02	31.96	30.01	28.20
5.4	24.16	26.36	28.72	31.20	33.77	36.34	38.84	41.13	43.04	44.37	44.95	44.68	43.66	42.05	40.06	37.85	35.56	33.27	31.08	29.02	27.13
5.6	24.04	26.24	28.60	31.09	33.63	36.15	38.54	40.69	42.42	43.58	44.03	43.70	42.65	41.02	38.98	36.69	34.27	31.86	29.53	27.36	25.41
5.8	23.61	25.79	28.14	30.60	33.10	35.54	37.82	39.80	41.35	42.33	42.63	42.22	41.13	39.46	37.36	34.96	32.42	29.85	27.38	25.11	23.12
6	22.93	25.07	27.38	29.81	32.26	34.62	36.78	38.61	39.98	40.80	40.96	40.45	39.28	37.54	35.34	32.82	30.12	27.38	24.76	22.38	20.34
6.2	22.03	24.11	26.40	28.80	31.20	33.48	35.53	37.22	38.45	39.11	39.14	38.51	37.25	35.41	33.09	30.42	27.54	24.61	21.80	19.29	17.23
6.4	20.99	23.02	25.28	27.65	30.01	32.22	34.17	35.75	36.84	37.36	37.26	36.52	35.15	33.20	30.75	27.92	24.85	21.70	18.70	16.05	13.95
6.6	19.94	21.90	24.12	26.46	28.77	30.92	32.78	34.24	35.21	35.62	35.40	34.56	33.08	31.03	28.46	25.48	22.23	18.90	15.70	12.92	10.79
6.8	19.00	20.88	23.05	25.34	27.59	29.66	31.42	32.77	33.62	33.92	33.61	32.67	31.12	28.98	26.33	23.26	19.89	16.42	13.08	10.20	8.07
7	18.31	20.10	22.18	24.38	26.53	28.48	30.13	31.36	32.11	32.31	31.91	30.91	29.31	27.14	24.46	21.36	17.97	14.47	11.11	8.22	6.12

Table 52 - Radial basis functions determined by *MATLAB*® for polyunsaturates mass iron vs time; where iron amount 5 means 2.49 mg/l, 10 = 4.98 mg/l and 15 = 7.47 mg/l (Figure 3-13d)

Polyunsaturates / mg	**Iron**																				
Time / days	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
3	8.27	8.63	8.96	9.27	9.53	9.75	9.90	9.98	9.97	9.88	9.70	9.43	9.07	8.64	8.14	7.59	7.00	6.39	5.76	5.15	4.55
3.2	10.38	10.89	11.39	11.87	12.31	12.69	12.99	13.20	13.30	13.27	13.11	12.81	12.39	11.85	11.22	10.52	9.76	8.97	8.18	7.40	6.65
3.4	12.54	13.22	13.90	14.57	15.21	15.78	16.26	16.63	16.85	16.90	16.76	16.44	15.94	15.28	14.49	13.61	12.66	11.68	10.70	9.74	8.82
3.6	14.71	15.57	16.45	17.33	18.18	18.97	19.66	20.21	20.58	20.72	20.62	20.27	19.68	18.88	17.92	16.83	15.67	14.48	13.30	12.15	11.06
3.8	16.86	17.91	19.00	20.10	21.18	22.20	23.12	23.88	24.42	24.68	24.63	24.25	23.55	22.60	21.43	20.12	18.74	17.33	15.93	14.59	13.33
4	18.96	20.19	21.48	22.80	24.12	25.39	26.57	27.56	28.30	28.69	28.70	28.28	27.47	26.33	24.95	23.42	21.80	20.17	18.57	17.04	15.60
4.2	20.97	22.37	23.85	25.38	26.93	28.45	29.88	31.13	32.08	32.64	32.71	32.26	31.32	29.99	28.39	26.62	24.78	22.94	21.15	19.45	17.86
4.4	22.85	24.40	26.04	27.76	29.52	31.27	32.95	34.45	35.64	36.37	36.51	36.03	34.96	33.44	31.62	29.64	27.60	25.58	23.63	21.79	20.07
4.6	24.58	26.24	28.01	29.87	31.80	33.74	35.63	37.36	38.77	39.68	39.91	39.39	38.20	36.51	34.52	32.37	30.18	28.03	25.97	24.03	22.22
4.8	26.12	27.85	29.70	31.66	33.69	35.76	37.79	39.68	41.26	42.31	42.62	42.10	40.83	39.04	36.94	34.71	32.45	30.24	28.13	26.14	24.29
5	27.47	29.23	31.10	33.07	35.14	37.24	39.32	41.28	42.93	44.05	44.41	43.91	42.65	40.87	38.79	36.58	34.35	32.17	30.09	28.12	26.27
5.2	28.62	30.35	32.18	34.11	36.11	38.16	40.18	42.07	43.67	44.77	45.15	44.73	43.59	41.94	40.01	37.95	35.86	33.81	31.83	29.94	28.15
5.4	29.56	31.22	32.96	34.77	36.64	38.53	40.39	42.12	43.57	44.57	44.95	44.63	43.69	42.30	40.64	38.83	36.99	35.15	33.36	31.62	29.93
5.6	30.32	31.86	33.45	35.09	36.76	38.43	40.05	41.54	42.79	43.66	44.03	43.84	43.15	42.08	40.75	39.29	37.77	36.23	34.69	33.15	31.62
5.8	30.90	32.30	33.70	35.11	36.53	37.93	39.28	40.52	41.55	42.28	42.63	42.58	42.15	41.41	40.47	39.40	38.26	37.07	35.84	34.56	33.22
6	31.34	32.56	33.74	34.90	36.04	37.15	38.21	39.18	40.00	40.61	40.96	41.03	40.85	40.45	39.90	39.24	38.51	37.72	36.83	35.84	34.71
6.2	31.63	32.66	33.61	34.50	35.34	36.15	36.93	37.65	38.28	38.78	39.14	39.33	39.37	39.29	39.11	38.87	38.56	38.17	37.67	36.99	36.10
6.4	31.78	32.62	33.33	33.94	34.49	35.02	35.53	36.02	36.48	36.90	37.26	37.56	37.81	38.01	38.18	38.32	38.42	38.45	38.32	37.97	37.32
6.6	31.77	32.42	32.89	33.24	33.52	33.79	34.06	34.36	34.69	35.03	35.40	35.80	36.22	36.66	37.13	37.62	38.10	38.50	38.74	38.71	38.29
6.8	31.55	32.02	32.28	32.40	32.46	32.51	32.59	32.73	32.94	33.23	33.61	34.08	34.64	35.28	36.00	36.77	37.55	38.28	38.84	39.08	38.88
7	31.06	31.39	31.49	31.43	31.31	31.19	31.13	31.15	31.28	31.53	31.91	32.44	33.10	33.90	34.80	35.77	36.78	37.74	38.52	38.98	38.96

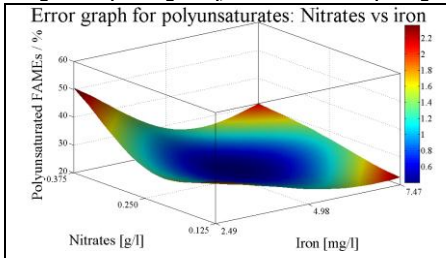
Table 53 - Radial basis functions determined by *MATLAB*® for polyunsaturates mass iron vs phosphates; where iron amount 5 means 2.49 mg/l, 10 = 4.98 mg/l and 15 = 7.47 mg/l; where phosphate amount 5 means a ratio of K_2HPO_4:KH_2PO_4 of 0.0375:0.0875 g/l, 10 = 0.0750:0.1750 g/l and 15 = 0.1125:0.2625 g/l (Figure 3-13e)

Polyunsaturates / mg	**Iron**																				
Phosphates	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
5	35.74	36.76	37.71	38.59	39.36	40.00	40.47	40.75	40.81	40.62	40.16	39.44	38.47	37.25	35.83	34.25	32.55	30.79	29.03	27.35	25.81
5.5	35.63	36.72	37.76	38.72	39.59	40.32	40.88	41.25	41.37	41.23	40.80	40.09	39.09	37.84	36.37	34.73	32.97	31.15	29.34	27.60	26.01
6	35.41	36.58	37.71	38.77	39.74	40.58	41.25	41.72	41.93	41.86	41.47	40.76	39.75	38.47	36.95	35.26	33.44	31.58	29.72	27.94	26.30
6.5	35.06	36.32	37.55	38.72	39.81	40.78	41.58	42.17	42.49	42.50	42.16	41.48	40.45	39.12	37.55	35.80	33.94	32.03	30.13	28.32	26.62
7	34.58	35.93	37.25	38.55	39.77	40.89	41.84	42.59	43.04	43.16	42.89	42.22	41.17	39.80	38.16	36.35	34.42	32.46	30.52	28.67	26.93
7.5	33.94	35.37	36.81	38.22	39.60	40.88	42.02	42.95	43.57	43.82	43.63	42.98	41.91	40.46	38.74	36.84	34.85	32.83	30.85	28.95	27.17
8	33.11	34.63	36.17	37.72	39.25	40.72	42.06	43.20	44.03	44.44	44.35	43.72	42.60	41.06	39.24	37.25	35.17	33.09	31.06	29.12	27.30
8.5	32.07	33.66	35.31	36.99	38.68	40.34	41.91	43.28	44.34	44.94	44.96	44.35	43.16	41.52	39.59	37.49	35.34	33.20	31.12	29.15	27.30
9	30.79	32.46	34.20	36.00	37.84	39.68	41.46	43.07	44.37	45.17	45.31	44.72	43.47	41.73	39.69	37.51	35.29	33.10	31.00	29.00	27.13
9.5	29.26	30.98	32.80	34.70	36.67	38.66	40.62	42.44	43.95	44.94	45.20	44.64	43.35	41.55	39.45	37.22	34.97	32.77	30.66	28.66	26.79
10	27.47	29.23	31.10	33.07	35.14	37.24	39.32	41.28	42.93	44.05	44.41	43.91	42.65	40.87	38.79	36.58	34.35	32.17	30.09	28.12	26.27
10.5	25.42	27.19	29.09	31.11	33.23	35.40	37.54	39.54	41.24	42.41	42.85	42.45	41.31	39.65	37.67	35.56	33.41	31.31	29.28	27.36	25.56
11	23.11	24.87	26.79	28.84	30.98	33.17	35.31	37.30	38.97	40.13	40.62	40.35	39.40	37.93	36.15	34.20	32.18	30.18	28.25	26.40	24.66
11.5	20.57	22.32	24.23	26.29	28.45	30.62	32.74	34.67	36.29	37.43	37.96	37.83	37.09	35.86	34.30	32.55	30.70	28.84	27.01	25.26	23.60
12	17.84	19.56	21.47	23.54	25.69	27.86	29.94	31.83	33.40	34.52	35.10	35.11	34.56	33.57	32.24	30.70	29.03	27.31	25.61	23.96	22.40
12.5	14.96	16.65	18.56	20.64	22.81	24.97	27.04	28.90	30.44	31.57	32.21	32.33	31.97	31.19	30.07	28.72	27.22	25.65	24.07	22.54	21.07
13	12.01	13.66	15.58	17.69	19.88	22.07	24.13	25.98	27.52	28.67	29.37	29.61	29.40	28.80	27.87	26.69	25.35	23.92	22.46	21.02	19.66
13.5	9.08	10.70	12.63	14.78	17.02	19.23	21.31	23.17	24.72	25.90	26.66	27.00	26.92	26.46	25.69	24.67	23.46	22.15	20.80	19.46	18.20
14	6.29	7.88	9.83	12.01	14.29	16.54	18.63	20.50	22.07	23.28	24.10	24.52	24.55	24.22	23.58	22.68	21.60	20.40	19.15	17.91	16.74
14.5	3.79	5.35	7.30	9.51	11.81	14.06	16.16	18.03	19.60	20.84	21.70	22.19	22.30	22.08	21.55	20.78	19.81	18.71	17.55	16.40	15.33
15	1.75	3.25	5.18	7.35	9.62	11.84	13.91	15.76	17.33	18.57	19.47	20.01	20.20	20.06	19.64	18.97	18.11	17.12	16.06	15.00	14.02

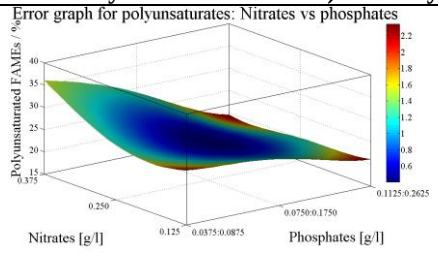
Table 54 - Radial basis functions determined by *MATLAB*® for polyunsaturates mass phosphates vs time; where phosphate amount 5 means a ratio of K_2HPO_4:KH_2PO_4 of 0.0375:0.0875 g/l, 10 = 0.0750:0.1750 g/l and 15 = 0.1125:0.2625 g/l (Figure 3-13f)

Polyunsaturates / mg	**Phosphates**																				
Time / days	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
3	3.46	3.71	4.32	5.15	6.08	6.99	7.83	8.55	9.11	9.50	9.70	9.71	9.55	9.21	8.71	8.07	7.33	6.51	5.66	4.82	4.04
3.2	6.62	6.95	7.61	8.49	9.46	10.41	11.29	12.02	12.59	12.95	13.11	13.04	12.77	12.30	11.66	10.87	9.97	8.99	7.99	7.00	6.07
3.4	10.25	10.64	11.33	12.22	13.20	14.16	15.05	15.79	16.34	16.67	16.76	16.60	16.20	15.58	14.77	13.81	12.74	11.59	10.43	9.28	8.20
3.6	14.18	14.63	15.34	16.23	17.20	18.17	19.05	19.79	20.33	20.62	20.62	20.34	19.79	18.99	17.99	16.83	15.56	14.24	12.90	11.59	10.35
3.8	18.26	18.78	19.51	20.39	21.36	22.32	23.21	23.95	24.47	24.71	24.63	24.21	23.48	22.46	21.23	19.85	18.37	16.84	15.32	13.83	12.42
4	22.36	22.94	23.70	24.58	25.54	26.51	27.41	28.16	28.68	28.87	28.70	28.12	27.17	25.91	24.42	22.78	21.06	19.32	17.59	15.93	14.34
4.2	26.36	27.01	27.78	28.68	29.64	30.62	31.53	32.31	32.83	32.99	32.71	31.95	30.76	29.22	27.44	25.52	23.54	21.57	19.64	17.80	16.04
4.4	30.20	30.88	31.68	32.57	33.52	34.50	35.44	36.23	36.77	36.90	36.51	35.56	34.10	32.25	30.15	27.94	25.71	23.51	21.39	19.36	17.45
4.6	33.81	34.51	35.29	36.16	37.09	38.04	38.96	39.77	40.30	40.40	39.91	38.74	36.99	34.82	32.42	29.93	27.45	25.05	22.75	20.57	18.52
4.8	37.14	37.83	38.57	39.38	40.23	41.11	41.98	42.73	43.22	43.25	42.62	41.25	39.23	36.78	34.11	31.38	28.70	26.11	23.66	21.36	19.20
5	40.16	40.80	41.47	42.16	42.89	43.63	44.35	44.96	45.31	45.20	44.41	42.85	40.62	37.96	35.10	32.21	29.37	26.66	24.10	21.70	19.47
5.2	42.87	43.42	43.96	44.50	45.03	45.56	46.04	46.40	46.50	46.15	45.15	43.43	41.08	38.32	35.36	32.37	29.46	26.67	24.04	21.59	19.31
5.4	45.25	45.69	46.06	46.39	46.67	46.91	47.07	47.09	46.84	46.18	44.95	43.08	40.67	37.89	34.92	31.91	28.97	26.15	23.49	21.02	18.74
5.6	47.33	47.62	47.79	47.86	47.84	47.74	47.52	47.15	46.51	45.51	44.03	42.03	39.59	36.82	33.88	30.90	27.97	25.15	22.50	20.04	17.78
5.8	49.12	49.24	49.18	48.96	48.61	48.13	47.52	46.73	45.70	44.36	42.63	40.51	38.03	35.28	32.39	29.45	26.55	23.76	21.13	18.70	16.48
6	50.65	50.57	50.26	49.73	49.03	48.17	47.15	45.97	44.57	42.91	40.96	38.70	36.18	33.44	30.58	27.68	24.82	22.06	19.46	17.07	14.91
6.2	51.92	51.64	51.06	50.22	49.16	47.92	46.52	44.97	43.24	41.30	39.14	36.75	34.17	31.43	28.59	25.72	22.89	20.15	17.58	15.23	13.14
6.4	52.91	52.44	51.59	50.43	49.03	47.44	45.69	43.81	41.79	39.61	37.26	34.76	32.12	29.36	26.54	23.69	20.87	18.16	15.61	13.29	11.28
6.6	53.59	52.93	51.82	50.36	48.65	46.75	44.70	42.55	40.28	37.90	35.40	32.81	30.11	27.34	24.52	21.69	18.90	16.20	13.67	11.39	9.44
6.8	53.86	53.03	51.70	49.99	48.02	45.86	43.58	41.21	38.75	36.21	33.61	30.94	28.21	25.43	22.63	19.83	17.07	14.39	11.89	9.65	7.78
7	53.65	52.67	51.17	49.29	47.13	44.80	42.36	39.83	37.23	34.60	31.91	29.20	26.45	23.69	20.92	18.17	15.46	12.84	10.40	8.23	6.43

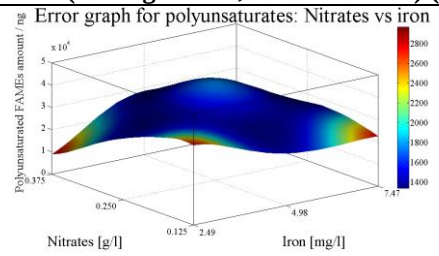
Table 55 - Error graphs for polyunsaturated FAME content derived from algal cells: (1) percentage and (2) mass produced (Nitrates – $NaNO_3$, phosphates – K_2HPO_4:KH_2PO_4, iron – $FeSO_4.7H_2O$, time – days after inoculation) with key to error (red high error, blue low error) (Figure 3-12, Figure 3-13)



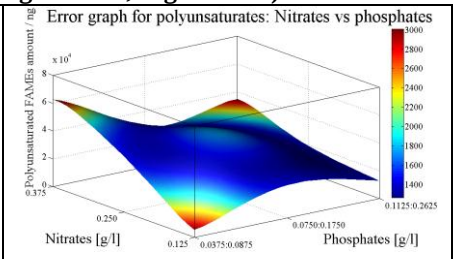
1a



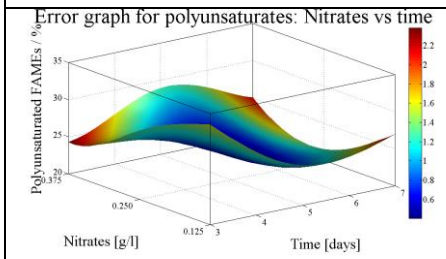
1b



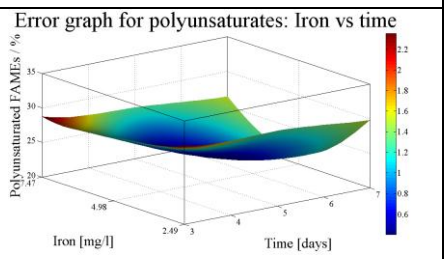
2a



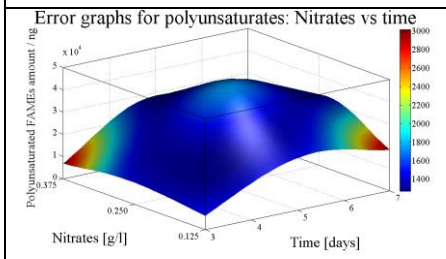
2b



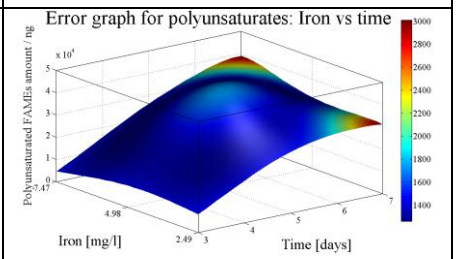
1c



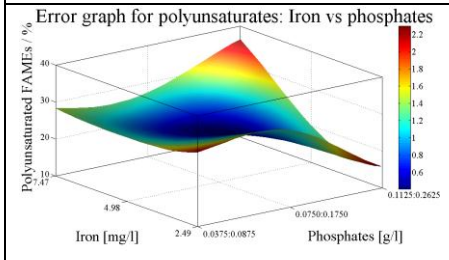
1d



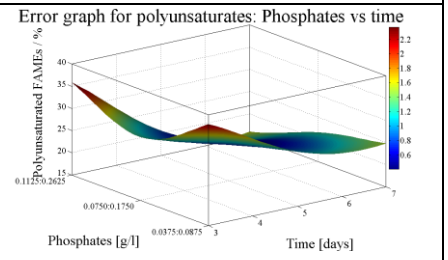
2c



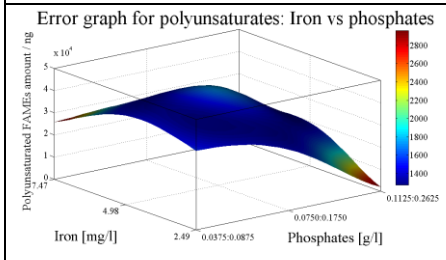
2d



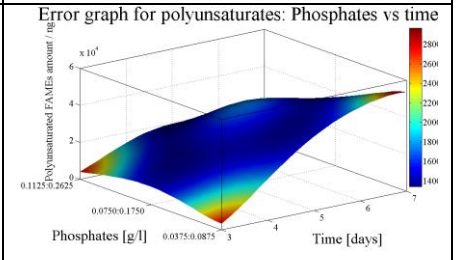
1e



1f



2e



2f

Table 56 - Radial basis functions determined by *MATLAB*® for saturates nitrates vs iron; where nitrates amount 5 means 0.125 g/l, 10 = 0.250 g/l and 15 = 0.375g/l; where iron amount 5 means 2.49 mg/l, 10 = 4.98 mg/l and 15 = 7.47 mg/l (Figure 3-14a)

Saturates / %	**Nitrates**																				
Iron	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
5	40.75	40.16	39.43	38.59	37.65	36.66	35.67	34.71	33.84	33.12	32.58	32.28	32.26	32.52	33.05	33.81	34.65	35.28	35.22	33.66	29.69
5.5	38.51	38.53	38.35	38.00	37.52	36.93	36.29	35.64	35.03	34.52	34.17	34.02	34.11	34.47	35.08	35.89	36.76	37.42	37.38	35.90	32.17
6	36.66	37.26	37.62	37.74	37.67	37.43	37.08	36.66	36.23	35.85	35.57	35.43	35.49	35.76	36.22	36.83	37.42	37.75	37.38	35.68	32.04
6.5	35.23	36.41	37.27	37.84	38.14	38.20	38.09	37.83	37.50	37.15	36.83	36.60	36.49	36.51	36.66	36.86	36.98	36.79	35.90	33.88	30.30
7	34.25	36.00	37.35	38.33	38.96	39.28	39.34	39.19	38.89	38.49	38.05	37.61	37.22	36.89	36.60	36.30	35.85	35.07	33.67	31.34	27.82
7.5	33.75	36.06	37.89	39.25	40.18	40.71	40.89	40.78	40.44	39.92	39.29	38.58	37.84	37.09	36.30	35.45	34.42	33.07	31.21	28.64	25.19
8	33.77	36.64	38.92	40.63	41.82	42.51	42.77	42.65	42.21	41.52	40.64	39.61	38.47	37.26	35.97	34.56	32.98	31.14	28.90	26.15	22.80
8.5	34.34	37.75	40.47	42.51	43.91	44.73	45.01	44.83	44.25	43.34	42.16	40.78	39.23	37.56	35.76	33.85	31.78	29.51	26.97	24.11	20.88
9	35.46	39.42	42.55	44.89	46.47	47.36	47.61	47.32	46.55	45.39	43.90	42.15	40.20	38.08	35.83	33.46	30.97	28.35	25.59	22.66	19.56
9.5	37.15	41.65	45.18	47.77	49.49	50.39	50.57	50.13	49.13	47.69	45.87	43.76	41.41	38.88	36.22	33.46	30.63	27.75	24.83	21.88	18.93
10	39.39	44.43	48.33	51.14	52.93	53.80	53.85	53.20	51.95	50.21	48.06	45.60	42.88	39.99	36.98	33.91	30.81	27.74	24.72	21.81	19.02
10.5	42.16	47.73	51.97	54.94	56.75	57.51	57.37	56.47	54.94	52.88	50.41	47.61	44.58	41.38	38.08	34.76	31.48	28.29	25.25	22.41	19.80
11	45.37	51.47	56.01	59.08	60.82	61.41	61.02	59.82	57.97	55.60	52.81	49.72	46.41	42.96	39.46	35.97	32.58	29.35	26.34	23.62	21.23
11.5	48.94	55.56	60.33	63.41	65.00	65.32	64.62	63.09	60.90	58.22	55.15	51.80	48.27	44.65	41.01	37.44	34.01	30.81	27.90	25.35	23.20
12	52.70	59.81	64.75	67.73	69.06	69.03	67.95	66.05	63.53	60.55	57.24	53.69	50.01	46.29	42.60	39.02	35.65	32.55	29.81	27.47	25.61
12.5	56.42	63.98	68.99	71.75	72.69	72.23	70.72	68.44	65.60	62.38	58.88	55.22	51.47	47.73	44.07	40.58	37.34	34.43	31.91	29.86	28.31
13	59.71	67.69	72.64	75.04	75.50	74.53	72.58	69.95	66.86	63.46	59.86	56.17	52.45	48.79	45.27	41.96	38.94	36.29	34.07	32.35	31.17
13.5	62.00	70.33	75.10	77.03	76.94	75.48	73.14	70.24	66.99	63.53	59.96	56.35	52.78	49.31	46.03	42.99	40.29	37.98	36.14	34.81	34.06
14	62.33	70.98	75.50	76.95	76.37	74.54	71.97	68.97	65.73	62.36	58.96	55.58	52.28	49.13	46.19	43.54	41.24	39.36	37.97	37.12	36.84
14.5	59.29	68.29	72.70	73.89	73.11	71.20	68.68	65.82	62.81	59.74	56.67	53.68	50.80	48.09	45.63	43.46	41.67	40.32	39.46	39.14	39.42
15	51.29	60.83	65.58	67.06	66.60	65.07	62.97	60.57	58.05	55.50	52.97	50.53	48.22	46.10	44.23	42.67	41.49	40.75	40.50	40.80	41.68

Table 57 - Radial basis functions determined by *MATLAB*® for saturates nitrates vs phosphates; where nitrates amount 5 means 0.125 g/l, 10 = 0.250 g/l and 15 = 0.375g/l; where phosphates of K_2HPO_4:KH_2PO_4 of 0.0375:0.0875 g/l, 10 = 0.0750:0.1750 g/l and 15 = 0.1125:0.2625 g/l (Figure 3-14b)

Saturates / %	**Nitrates**																				
Phosphates	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
5	15.42	20.70	25.26	29.10	32.24	34.73	36.59	37.90	38.69	39.03	38.97	38.58	37.91	37.00	35.92	34.72	33.43	32.11	30.79	29.51	28.32
5.5	18.04	23.33	27.84	31.58	34.57	36.87	38.51	39.57	40.09	40.14	39.79	39.09	38.11	36.90	35.52	34.01	32.42	30.80	29.20	27.64	26.17
6	20.94	26.23	30.68	34.31	37.16	39.26	40.68	41.48	41.73	41.50	40.85	39.86	38.58	37.07	35.39	33.59	31.72	29.83	27.95	26.13	24.40
6.5	24.01	29.29	33.68	37.20	39.88	41.78	42.97	43.52	43.51	43.00	42.07	40.79	39.22	37.44	35.48	33.41	31.28	29.12	26.99	24.93	22.96
7	27.12	32.39	36.71	40.10	42.62	44.33	45.30	45.60	45.33	44.55	43.35	41.80	39.97	37.92	35.71	33.39	31.02	28.63	26.28	23.99	21.81
7.5	30.14	35.38	39.63	42.90	45.27	46.78	47.53	47.60	47.08	46.06	44.61	42.82	40.75	38.46	36.02	33.49	30.90	28.31	25.75	23.27	20.91
8	32.92	38.13	42.31	45.47	47.68	49.01	49.56	49.42	48.68	47.44	45.77	43.76	41.48	38.99	36.36	33.63	30.86	28.10	25.37	22.73	20.21
8.5	35.34	40.52	44.62	47.67	49.74	50.91	51.29	50.96	50.03	48.60	46.75	44.55	42.10	39.45	36.65	33.78	30.86	27.96	25.11	22.34	19.70
9	37.28	42.42	46.45	49.40	51.35	52.39	52.62	52.14	51.06	49.48	47.48	45.15	42.56	39.78	36.87	33.89	30.87	27.87	24.92	22.07	19.34
9.5	38.65	43.75	47.71	50.58	52.43	53.37	53.49	52.90	51.71	50.02	47.93	45.50	42.83	39.97	36.99	33.94	30.86	27.80	24.80	21.89	19.12
10	39.39	44.43	48.33	51.14	52.93	53.80	53.85	53.20	51.95	50.21	48.06	45.60	42.88	39.99	36.98	33.91	30.81	27.74	24.72	21.81	19.02
10.5	39.48	44.47	48.32	51.09	52.84	53.68	53.71	53.04	51.78	50.03	47.88	45.42	42.72	39.85	36.85	33.80	30.73	27.68	24.70	21.81	19.05
11	38.96	43.88	47.69	50.44	52.19	53.03	53.08	52.44	51.22	49.51	47.41	45.00	42.36	39.55	36.62	33.63	30.63	27.65	24.74	21.92	19.23
11.5	37.89	42.74	46.52	49.26	51.03	51.92	52.02	51.45	50.31	48.69	46.69	44.38	41.84	39.13	36.32	33.44	30.54	27.67	24.87	22.15	19.56
12	36.37	41.16	44.91	47.66	49.47	50.43	50.62	50.16	49.13	47.64	45.78	43.61	41.22	38.66	35.99	33.26	30.51	27.78	25.12	22.54	20.09
12.5	34.54	39.26	42.99	45.76	47.63	48.67	48.99	48.66	47.79	46.46	44.76	42.77	40.56	38.18	35.69	33.15	30.58	28.03	25.54	23.13	20.85
13	32.55	37.19	40.90	43.70	45.63	46.79	47.23	47.06	46.37	45.23	43.73	41.94	39.94	37.78	35.50	33.17	30.81	28.47	26.18	23.97	21.87
13.5	30.53	35.10	38.79	41.61	43.63	44.90	45.49	45.50	44.99	44.06	42.79	41.23	39.46	37.53	35.49	33.39	31.27	29.16	27.09	25.10	23.22
14	28.64	33.13	36.79	39.65	41.75	43.14	43.89	44.08	43.78	43.07	42.03	40.72	39.20	37.53	35.74	33.90	32.03	30.16	28.34	26.59	24.94
14.5	26.99	31.41	35.05	37.94	40.12	41.64	42.55	42.93	42.84	42.37	41.57	40.51	39.26	37.85	36.33	34.75	33.14	31.54	29.98	28.48	27.08
15	25.72	30.06	33.69	36.61	38.87	40.51	41.58	42.15	42.28	42.04	41.50	40.70	39.71	38.57	37.33	36.03	34.69	33.36	32.06	30.82	29.68

Table 58 - Radial basis functions determined by *MATLAB*® for saturates nitrates vs time; where nitrates amount 5 means 0.125 g/l, 10 = 0.250 g/l and 15 = 0.375g/l (Figure 3-14c)

Saturates / %	**Nitrates**																				
Time	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
3	30.01	35.03	39.29	42.80	45.54	47.55	48.83	49.42	49.33	48.60	47.27	45.37	42.96	40.08	36.79	33.15	29.20	24.99	20.58	16.00	11.30
3.2	32.08	37.22	41.55	45.07	47.80	49.75	50.96	51.46	51.28	50.47	49.06	47.11	44.66	41.76	38.47	34.84	30.92	26.75	22.38	17.85	13.20
3.4	34.21	39.43	43.79	47.30	49.97	51.83	52.93	53.32	53.03	52.12	50.63	48.62	46.13	43.22	39.95	36.36	32.49	28.39	24.09	19.64	15.06
3.6	36.29	41.56	45.92	49.37	51.95	53.70	54.67	54.91	54.49	53.47	51.89	49.81	47.29	44.39	41.14	37.61	33.82	29.81	25.62	21.27	16.82
3.8	38.18	43.48	47.81	51.19	53.65	55.26	56.07	56.16	55.59	54.44	52.76	50.63	48.08	45.18	41.98	38.52	34.82	30.93	26.87	22.67	18.36
4	39.78	45.08	49.35	52.63	54.96	56.41	57.05	56.98	56.26	54.98	53.21	51.01	48.44	45.55	42.40	39.01	35.43	31.68	27.77	23.75	19.62
4.2	40.95	46.23	50.43	53.59	55.78	57.07	57.55	57.31	56.45	55.04	53.17	50.91	48.31	45.44	42.34	39.04	35.58	31.97	28.24	24.41	20.50
4.4	41.58	46.81	50.93	53.99	56.04	57.18	57.50	57.11	56.11	54.59	52.63	50.31	47.69	44.83	41.78	38.56	35.21	31.76	28.21	24.59	20.93
4.6	41.57	46.74	50.78	53.73	55.67	56.68	56.87	56.36	55.24	53.62	51.58	49.21	46.56	43.70	40.68	37.54	34.30	30.99	27.63	24.24	20.84
4.8	40.85	45.96	49.92	52.79	54.63	55.55	55.64	55.04	53.84	52.14	50.04	47.62	44.94	42.07	39.07	35.97	32.82	29.64	26.46	23.31	20.21
5	39.39	44.43	48.33	51.14	52.93	53.80	53.85	53.20	51.95	50.21	48.06	45.60	42.88	39.99	36.98	33.91	30.81	27.74	24.72	21.81	19.02
5.2	37.23	42.20	46.06	48.85	50.63	51.50	51.56	50.91	49.66	47.90	45.73	43.23	40.48	37.54	34.50	31.40	28.32	25.32	22.45	19.76	17.31
5.4	34.43	39.34	43.18	45.99	47.82	48.76	48.88	48.29	47.08	45.34	43.17	40.64	37.84	34.85	31.74	28.58	25.47	22.49	19.73	17.26	15.15
5.6	31.13	35.98	39.84	42.71	44.65	45.71	45.95	45.47	44.35	42.68	40.53	37.99	35.15	32.08	28.86	25.61	22.42	19.41	16.71	14.43	12.67
5.8	27.51	32.32	36.22	39.21	41.30	42.54	42.97	42.66	41.68	40.10	38.01	35.48	32.59	29.43	26.09	22.69	19.36	16.26	13.57	11.45	10.04
6	23.78	28.56	32.54	35.70	38.01	39.49	40.16	40.06	39.26	37.82	35.81	33.31	30.40	27.15	23.67	20.09	16.57	13.32	10.56	8.55	7.49
6.2	20.20	24.97	29.07	32.45	35.03	36.79	37.74	37.90	37.32	36.06	34.18	31.74	28.83	25.52	21.91	18.14	14.39	10.92	8.03	6.07	5.34
6.4	17.05	21.84	26.10	29.74	32.63	34.72	35.98	36.43	36.11	35.06	33.35	31.02	28.16	24.83	21.13	17.20	13.22	9.50	6.41	4.44	4.02
6.6	14.67	19.50	23.97	27.89	31.11	33.53	35.12	35.88	35.84	35.05	33.55	31.40	28.65	25.38	21.67	17.65	13.53	9.59	6.31	4.30	4.17
6.8	13.49	18.38	23.04	27.23	30.75	33.49	35.40	36.47	36.74	36.23	35.00	33.09	30.55	27.44	23.86	19.91	15.79	11.81	8.46	6.49	6.69
7	13.93	18.86	23.66	28.05	31.81	34.81	37.01	38.40	38.98	38.79	37.88	36.28	34.05	31.25	27.95	24.28	20.42	16.69	13.60	11.92	12.56

Table 59 - Radial basis functions determined by *MATLAB*® for saturates iron vs time; where iron amount 5 means 2.49 mg/l, 10 = 4.98 mg/l and 15 = 7.47 mg/l (Figure 3-14d)

Saturates / %	**Iron**																				
Time / days	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
3	24.46	26.99	29.45	31.85	34.19	36.49	38.74	40.96	43.13	45.24	47.27	49.17	50.88	52.35	53.47	54.12	54.15	53.35	51.41	47.93	42.44
3.2	25.06	27.69	30.24	32.73	35.16	37.54	39.90	42.24	44.56	46.84	49.06	51.18	53.13	54.84	56.20	57.08	57.33	56.70	54.90	51.52	46.14
3.4	25.87	28.57	31.17	33.70	36.17	38.60	41.02	43.44	45.85	48.26	50.63	52.91	55.04	56.94	58.47	59.51	59.86	59.29	57.50	54.12	48.82
3.6	26.84	29.55	32.16	34.68	37.15	39.58	42.01	44.46	46.93	49.42	51.89	54.29	56.56	58.59	60.25	61.38	61.78	61.21	59.38	56.00	50.78
3.8	27.88	30.57	33.13	35.61	38.02	40.41	42.80	45.24	47.72	50.24	52.76	55.25	57.61	59.74	61.49	62.69	63.12	62.55	60.72	57.36	52.27
4	28.94	31.55	34.03	36.40	38.72	41.01	43.33	45.70	48.15	50.66	53.21	55.74	58.16	60.37	62.19	63.45	63.93	63.40	61.60	58.32	53.39
4.2	29.94	32.43	34.78	37.01	39.17	41.33	43.53	45.80	48.17	50.64	53.17	55.72	58.18	60.44	62.33	63.66	64.21	63.75	62.05	58.90	54.16
4.4	30.82	33.16	35.32	37.37	39.35	41.33	43.36	45.49	47.75	50.14	52.63	55.17	57.65	59.94	61.89	63.30	63.94	63.60	62.03	59.06	54.54
4.6	31.57	33.69	35.64	37.46	39.22	40.98	42.81	44.77	46.89	49.16	51.58	54.08	56.55	58.88	60.89	62.38	63.13	62.92	61.53	58.76	54.50
4.8	32.15	34.02	35.71	37.27	38.77	40.29	41.89	43.65	45.59	47.73	50.04	52.47	54.93	57.26	59.32	60.89	61.76	61.70	60.50	57.97	53.97
5	32.58	34.17	35.57	36.83	38.05	39.29	40.64	42.16	43.90	45.87	48.06	50.41	52.81	55.15	57.24	58.88	59.86	59.96	58.96	56.67	52.97
5.2	32.92	34.18	35.25	36.20	37.10	38.05	39.12	40.39	41.91	43.69	45.73	47.97	50.30	52.61	54.72	56.42	57.51	57.76	56.96	54.93	51.53
5.4	33.24	34.15	34.87	35.48	36.05	36.68	37.46	38.45	39.73	41.30	43.17	45.27	47.52	49.78	51.89	53.64	54.82	55.21	54.61	52.83	49.75
5.6	33.65	34.19	34.54	34.79	35.02	35.32	35.79	36.49	37.51	38.86	40.53	42.48	44.62	46.82	48.90	50.68	51.94	52.46	52.05	50.53	47.77
5.8	34.31	34.45	34.42	34.31	34.18	34.14	34.28	34.69	35.43	36.54	38.01	39.79	41.80	43.92	45.96	47.75	49.07	49.71	49.48	48.21	45.77
6	35.36	35.10	34.69	34.20	33.71	33.33	33.15	33.25	33.71	34.56	35.81	37.41	39.29	41.30	43.29	45.07	46.44	47.19	47.13	46.09	43.96
6.2	36.97	36.32	35.52	34.66	33.82	33.10	32.59	32.39	32.56	33.16	34.18	35.59	37.31	39.21	41.13	42.89	44.28	45.12	45.21	44.40	42.56
6.4	39.32	38.27	37.11	35.89	34.70	33.65	32.82	32.32	32.22	32.55	33.35	34.56	36.11	37.89	39.72	41.44	42.84	43.74	43.96	43.36	41.80
6.6	42.54	41.13	39.61	38.06	36.55	35.19	34.06	33.27	32.90	32.99	33.55	34.56	35.94	37.57	39.30	40.96	42.34	43.29	43.61	43.18	41.87
6.8	46.80	45.05	43.21	41.34	39.54	37.89	36.49	35.44	34.81	34.66	35.00	35.81	37.00	38.48	40.08	41.65	43.00	43.95	44.36	44.07	42.97
7	52.21	50.15	48.02	45.88	43.82	41.92	40.28	38.99	38.13	37.76	37.88	38.48	39.48	40.79	42.25	43.70	44.98	45.92	46.36	46.18	45.26

Table 60 - Radial basis functions determined by *MATLAB*® for saturates iron vs phosphates; where iron amount 5 means 2.49 mg/l, 10 = 4.98 mg/l and 15 = 7.47 mg/l; where phosphate amount 5 means a ratio of K_2HPO_4:KH_2PO_4 of 0.0375:0.0875 g/l, 10 = 0.0750:0.1750 g/l and 15 = 0.1125:0.2625 g/l (Figure 3-14e)

Saturates / %	**Iron**																				
Phosphates	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
5	25.80	27.42	28.93	30.35	31.70	32.99	34.25	35.48	36.69	37.86	38.97	39.98	40.81	41.40	41.67	41.50	40.81	39.50	37.46	34.64	30.96
5.5	25.77	27.45	29.02	30.48	31.88	33.22	34.55	35.87	37.19	38.51	39.79	41.00	42.05	42.89	43.39	43.46	42.99	41.85	39.95	37.19	33.52
6	26.11	27.85	29.45	30.94	32.36	33.74	35.12	36.52	37.95	39.40	40.85	42.26	43.54	44.62	45.38	45.70	45.45	44.50	42.74	40.07	36.40
6.5	26.75	28.52	30.14	31.65	33.08	34.48	35.90	37.35	38.87	40.45	42.07	43.67	45.18	46.51	47.52	48.09	48.08	47.33	45.72	43.13	39.47
7	27.61	29.40	31.02	32.52	33.95	35.36	36.79	38.29	39.89	41.59	43.35	45.14	46.87	48.43	49.70	50.52	50.74	50.19	48.73	46.22	42.58
7.5	28.60	30.39	32.00	33.48	34.89	36.29	37.73	39.26	40.92	42.71	44.61	46.57	48.50	50.29	51.80	52.86	53.30	52.94	51.62	49.20	45.57
8	29.63	31.41	32.99	34.44	35.82	37.19	38.63	40.18	41.88	43.75	45.77	47.87	49.98	51.97	53.69	54.96	55.60	55.42	54.23	51.88	48.26
8.5	30.61	32.36	33.91	35.32	36.66	38.00	39.42	40.97	42.71	44.64	46.75	48.97	51.22	53.37	55.26	56.72	57.52	57.48	56.40	54.11	50.48
9	31.47	33.18	34.68	36.05	37.34	38.65	40.04	41.60	43.35	45.32	47.48	49.78	52.14	54.41	56.43	58.01	58.93	58.99	57.98	55.72	52.08
9.5	32.14	33.79	35.25	36.56	37.81	39.08	40.46	42.00	43.75	45.73	47.93	50.27	52.68	55.01	57.10	58.75	59.73	59.84	58.86	56.60	52.94
10	32.58	34.17	35.57	36.83	38.05	39.29	40.64	42.16	43.90	45.87	48.06	50.41	52.81	55.15	57.24	58.88	59.86	59.96	58.96	56.67	52.97
10.5	32.79	34.30	35.63	36.85	38.03	39.25	40.58	42.08	43.79	45.74	47.88	50.18	52.53	54.80	56.82	58.40	59.31	59.33	58.26	55.91	52.15
11	32.77	34.19	35.46	36.64	37.80	39.00	40.30	41.78	43.45	45.34	47.41	49.62	51.86	54.00	55.89	57.32	58.09	57.98	56.79	54.34	50.50
11.5	32.59	33.91	35.11	36.25	37.39	38.57	39.86	41.30	42.92	44.72	46.69	48.76	50.84	52.80	54.49	55.72	56.28	55.99	54.63	52.05	48.13
12	32.32	33.51	34.65	35.76	36.87	38.04	39.31	40.70	42.25	43.95	45.78	47.67	49.55	51.28	52.72	53.69	54.00	53.47	51.91	49.18	45.15
12.5	32.04	33.12	34.18	35.25	36.35	37.50	38.74	40.08	41.53	43.10	44.76	46.45	48.07	49.53	50.68	51.35	51.37	50.57	48.79	45.88	41.74
13	31.90	32.83	33.82	34.85	35.93	37.06	38.25	39.51	40.85	42.27	43.73	45.17	46.53	47.68	48.50	48.84	48.54	47.45	45.42	42.32	38.06
13.5	32.01	32.79	33.70	34.69	35.74	36.82	37.95	39.11	40.32	41.55	42.79	43.96	45.01	45.83	46.31	46.30	45.67	44.28	41.99	38.69	34.30
14	32.55	33.17	33.99	34.92	35.91	36.92	37.95	38.99	40.02	41.05	42.03	42.92	43.65	44.12	44.24	43.87	42.90	41.19	38.64	35.14	30.63
14.5	33.72	34.17	34.86	35.69	36.58	37.48	38.37	39.24	40.07	40.86	41.57	42.15	42.54	42.66	42.41	41.68	40.36	38.35	35.54	31.84	27.19
15	35.74	35.97	36.49	37.16	37.88	38.61	39.31	39.97	40.57	41.09	41.50	41.76	41.80	41.55	40.93	39.85	38.19	35.87	32.80	28.91	24.13

Table 61 - Radial basis functions determined by *MATLAB*® for saturates phosphates vs time; where phosphate amount 5 means a ratio of K_2HPO_4:KH_2PO_4 of 0.0375:0.0875 g/l, 10 = 0.0750:0.1750 g/l and 15 = 0.1125:0.2625 g/l (Figure 3-14f)

Saturates / %	**Phosphates**																				
Time / days	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
3	41.47	42.16	43.07	44.11	45.18	46.19	47.03	47.63	47.90	47.79	47.27	46.32	44.97	43.27	41.28	39.09	36.80	34.51	32.34	30.38	28.75
3.2	41.49	42.41	43.56	44.83	46.12	47.33	48.36	49.11	49.52	49.51	49.06	48.16	46.83	45.12	43.09	40.85	38.50	36.13	33.88	31.84	30.12
3.4	41.54	42.66	44.01	45.47	46.94	48.32	49.50	50.39	50.91	51.00	50.63	49.78	48.47	46.77	44.74	42.47	40.08	37.68	35.38	33.30	31.54
3.6	41.57	42.85	44.34	45.95	47.57	49.08	50.39	51.40	52.02	52.19	51.89	51.09	49.83	48.15	46.14	43.88	41.49	39.09	36.79	34.71	32.96
3.8	41.53	42.92	44.52	46.23	47.95	49.56	50.96	52.05	52.75	53.00	52.76	52.03	50.82	49.20	47.23	45.02	42.67	40.31	38.06	36.03	34.35
4	41.40	42.83	44.49	46.26	48.03	49.70	51.16	52.31	53.07	53.38	53.21	52.54	51.41	49.86	47.97	45.84	43.58	41.31	39.16	37.24	35.67
4.2	41.14	42.57	44.22	45.99	47.78	49.46	50.94	52.12	52.92	53.28	53.17	52.58	51.54	50.09	48.31	46.31	44.19	42.07	40.07	38.31	36.91
4.4	40.75	42.12	43.71	45.43	47.17	48.82	50.28	51.46	52.28	52.68	52.63	52.13	51.19	49.88	48.25	46.42	44.48	42.57	40.79	39.26	38.10
4.6	40.24	41.48	42.96	44.57	46.21	47.77	49.18	50.32	51.14	51.57	51.58	51.17	50.37	49.22	47.79	46.17	44.48	42.83	41.33	40.09	39.23
4.8	39.63	40.69	41.99	43.43	44.92	46.36	47.66	48.73	49.52	49.97	50.04	49.74	49.09	48.14	46.94	45.60	44.21	42.88	41.72	40.84	40.34
5	38.97	39.79	40.85	42.07	43.35	44.61	45.77	46.75	47.48	47.93	48.06	47.88	47.41	46.69	45.78	44.76	43.73	42.79	42.03	41.57	41.50
5.2	38.33	38.85	39.62	40.56	41.59	42.62	43.60	44.44	45.10	45.54	45.73	45.68	45.41	44.95	44.37	43.73	43.11	42.61	42.32	42.34	42.75
5.4	37.78	37.96	38.39	39.00	39.73	40.50	41.25	41.93	42.50	42.91	43.17	43.26	43.21	43.05	42.83	42.61	42.46	42.46	42.69	43.24	44.20
5.6	37.44	37.22	37.27	37.52	37.91	38.38	38.88	39.37	39.83	40.21	40.53	40.78	40.97	41.13	41.31	41.55	41.91	42.45	43.25	44.38	45.92
5.8	37.41	36.77	36.41	36.26	36.29	36.43	36.66	36.95	37.27	37.63	38.01	38.41	38.86	39.37	39.98	40.70	41.60	42.72	44.13	45.87	48.03
6	37.82	36.74	35.95	35.40	35.03	34.84	34.78	34.86	35.05	35.37	35.81	36.39	37.10	37.97	39.02	40.25	41.71	43.44	45.47	47.85	50.64
6.2	38.81	37.29	36.06	35.09	34.34	33.80	33.46	33.32	33.39	33.67	34.18	34.92	35.91	37.15	38.64	40.39	42.42	44.75	47.42	50.44	53.88
6.4	40.51	38.56	36.90	35.52	34.40	33.53	32.92	32.57	32.52	32.77	33.35	34.26	35.52	37.12	39.05	41.32	43.91	46.84	50.13	53.80	57.88
6.6	43.06	40.69	38.63	36.87	35.40	34.22	33.35	32.82	32.66	32.90	33.55	34.64	36.16	38.11	40.47	43.22	46.35	49.86	53.76	58.05	62.76
6.8	46.60	43.83	41.40	39.29	37.50	36.05	34.97	34.28	34.04	34.27	35.00	36.26	38.03	40.30	43.06	46.27	49.91	53.97	58.44	63.32	68.63
7	51.22	48.10	45.34	42.92	40.86	39.18	37.92	37.11	36.81	37.06	37.88	39.30	41.30	43.88	47.00	50.63	54.74	59.30	64.30	69.73	75.61

Table 62 - Radial basis functions determined by *MATLAB*® for saturates mass nitrates vs iron; where nitrates amount 5 means 0.125 g/l, 10 = 0.250 g/l and 15 = 0.375g/l; where iron amount 5 means 2.49 mg/l, 10 = 4.98 mg/l and 15 = 7.47 mg/l (Figure 3-15a)

Saturates / mg	**Nitrates**																				
Iron	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
5	30.83	32.63	33.89	34.92	35.80	36.53	37.05	37.28	37.16	36.60	35.57	34.04	32.00	29.47	26.48	23.09	19.37	15.41	11.36	7.48	4.30
5.5	31.28	33.39	35.03	36.44	37.70	38.80	39.67	40.22	40.36	40.01	39.12	37.64	35.59	32.98	29.87	26.33	22.45	18.34	14.17	10.21	6.92
6	31.18	33.65	35.78	37.69	39.44	41.00	42.31	43.25	43.73	43.65	42.93	41.53	39.48	36.80	33.57	29.91	25.91	21.72	17.53	13.60	10.22
6.5	30.90	33.71	36.33	38.78	41.07	43.17	44.99	46.41	47.29	47.52	47.01	45.72	43.65	40.88	37.53	33.73	29.62	25.36	21.15	17.19	13.70
7	30.60	33.75	36.81	39.79	42.65	45.33	47.72	49.68	51.05	51.65	51.38	50.18	48.07	45.18	41.66	37.68	33.42	29.06	24.77	20.73	17.09
7.5	30.38	33.82	37.30	40.77	44.19	47.48	50.49	53.07	54.99	56.03	56.02	54.89	52.69	49.59	45.83	41.62	37.16	32.65	28.24	24.08	20.27
8	30.25	33.96	37.81	41.74	45.69	49.57	53.24	56.49	59.05	60.61	60.89	59.79	57.39	53.99	49.90	45.39	40.70	36.00	31.44	27.13	23.16
8.5	30.25	34.19	38.34	42.66	47.09	51.53	55.84	59.81	63.10	65.29	65.91	64.75	62.02	58.18	53.67	48.82	43.87	38.97	34.26	29.81	25.70
9	30.39	34.50	38.89	43.51	48.32	53.22	58.11	62.77	66.86	69.80	70.82	69.50	66.25	61.85	56.88	51.70	46.51	41.45	36.61	32.07	27.86
9.5	30.70	34.90	39.42	44.23	49.27	54.48	59.77	64.97	69.78	73.52	74.99	73.38	69.51	64.57	59.24	53.82	48.49	43.34	38.44	33.85	29.59
10	31.17	35.39	39.94	44.78	49.88	55.18	60.60	66.00	71.10	75.25	77.00	75.22	71.04	65.90	60.47	55.02	49.69	44.57	39.70	35.13	30.88
10.5	31.82	35.98	40.43	45.16	50.12	55.26	60.49	65.65	70.43	74.19	75.71	74.19	70.43	65.63	60.46	55.23	50.08	45.11	40.37	35.90	31.73
11	32.65	36.66	40.92	45.39	50.03	54.78	59.54	64.11	68.17	71.14	72.26	71.10	68.08	63.97	59.33	54.52	49.70	44.99	40.48	36.19	32.16
11.5	33.67	37.46	41.41	45.50	49.67	53.88	57.99	61.82	65.06	67.29	68.05	67.14	64.75	61.35	57.34	53.04	48.65	44.30	40.07	36.03	32.19
12	34.86	38.38	41.94	45.54	49.15	52.71	56.10	59.16	61.65	63.26	63.73	62.94	61.01	58.19	54.77	51.01	47.09	43.13	39.24	35.47	31.88
12.5	36.22	39.41	42.53	45.57	48.53	51.39	54.05	56.39	58.20	59.30	59.52	58.78	57.15	54.79	51.88	48.61	45.14	41.59	38.06	34.60	31.26
13	37.72	40.56	43.16	45.58	47.87	50.01	51.97	53.63	54.86	55.53	55.52	54.78	53.36	51.33	48.83	46.00	42.95	39.80	36.61	33.46	30.39
13.5	39.31	41.77	43.80	45.55	47.14	48.59	49.89	50.95	51.68	51.97	51.75	50.99	49.70	47.93	45.76	43.30	40.63	37.83	34.98	32.14	29.33
14	40.89	42.92	44.32	45.38	46.29	47.11	47.81	48.35	48.65	48.63	48.24	47.43	46.23	44.65	42.75	40.59	38.25	35.78	33.24	30.68	28.13
14.5	42.15	43.73	44.49	44.90	45.22	45.49	45.71	45.83	45.78	45.50	44.95	44.11	42.96	41.53	39.85	37.95	35.88	33.70	31.43	29.13	26.83
15	42.44	43.66	43.95	43.92	43.82	43.70	43.56	43.35	43.03	42.56	41.89	41.02	39.92	38.61	37.09	35.40	33.57	31.63	29.62	27.55	25.48

Table 63 - Radial basis functions determined by *MATLAB*® for saturates mass nitrates vs phosphates; where nitrates amount 5 means 0.125 g/l, 10 = 0.250 g/l and 15 = 0.375g/l; where phosphates of K_2HPO_4:KH_2PO_4 of 0.0375:0.0875 g/l, 10 = 0.0750:0.1750 g/l and 15 = 0.1125:0.2625 g/l (Figure 3-15b)

Saturates/ mg	**Nitrates**																				
Phosphates	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
5	5.43	9.59	14.94	20.58	26.05	31.12	35.65	39.54	42.70	45.07	46.62	47.35	47.30	46.53	45.14	43.23	40.91	38.29	35.48	32.57	29.63
5.5	7.86	12.05	17.35	23.00	28.53	33.69	38.32	42.29	45.49	47.85	49.34	49.94	49.71	48.70	47.03	44.83	42.21	39.30	36.22	33.06	29.91
6	11.42	15.56	20.61	26.06	31.50	36.65	41.31	45.31	48.52	50.86	52.25	52.70	52.23	50.95	48.96	46.43	43.48	40.26	36.89	33.48	30.11
6.5	15.25	19.37	24.22	29.46	34.77	39.87	44.53	48.55	51.78	54.08	55.37	55.62	54.89	53.28	50.93	48.02	44.71	41.16	37.49	33.82	30.24
7	18.91	23.07	27.82	32.92	38.13	43.19	47.87	51.95	55.23	57.52	58.70	58.74	57.69	55.69	52.93	49.61	45.91	42.00	38.03	34.11	30.32
7.5	22.22	26.45	31.16	36.20	41.37	46.45	51.21	55.41	58.80	61.14	62.24	62.04	60.62	58.17	54.93	51.15	47.04	42.78	38.51	34.34	30.37
8	25.09	29.39	34.10	39.13	44.31	49.47	54.38	58.79	62.40	64.89	65.95	65.49	63.62	60.63	56.86	52.59	48.06	43.46	38.91	34.54	30.40
8.5	27.46	31.80	36.53	41.56	46.79	52.05	57.17	61.89	65.87	68.65	69.76	68.99	66.57	62.95	58.59	53.83	48.92	44.02	39.25	34.70	30.44
9	29.27	33.63	38.34	43.37	48.62	53.99	59.33	64.41	68.88	72.14	73.42	72.29	69.18	64.86	59.93	54.75	49.53	44.41	39.49	34.84	30.52
9.5	30.51	34.83	39.49	44.46	49.69	55.09	60.56	65.93	70.88	74.74	76.30	74.75	70.91	65.98	60.64	55.19	49.81	44.60	39.64	34.98	30.66
10	31.17	35.39	39.94	44.78	49.88	55.18	60.60	66.00	71.10	75.25	77.00	75.22	71.04	65.90	60.47	55.02	49.69	44.57	39.70	35.13	30.88
10.5	31.26	35.33	39.69	44.32	49.18	54.21	59.35	64.43	69.16	72.88	74.39	72.87	69.15	64.42	59.34	54.20	49.17	44.31	39.68	35.31	31.22
11	30.81	34.67	38.78	43.11	47.63	52.27	56.93	61.44	65.46	68.44	69.60	68.54	65.67	61.75	57.36	52.81	48.28	43.86	39.60	35.54	31.69
11.5	29.88	33.48	37.29	41.27	45.37	49.53	53.63	57.48	60.78	63.13	64.07	63.41	61.35	58.33	54.78	50.98	47.12	43.28	39.52	35.86	32.31
12	28.53	31.84	35.31	38.90	42.56	46.21	49.75	52.98	55.70	57.60	58.44	58.11	56.73	54.55	51.87	48.90	45.79	42.64	39.48	36.30	33.10
12.5	26.83	29.84	32.96	36.15	39.37	42.53	45.54	48.26	50.52	52.13	52.95	52.93	52.12	50.69	48.82	46.68	44.39	41.99	39.50	36.87	34.07
13	24.88	27.58	30.34	33.15	35.94	38.66	41.22	43.52	45.45	46.87	47.72	47.96	47.65	46.87	45.76	44.43	42.96	41.35	39.59	37.57	35.20
13.5	22.73	25.13	27.56	30.00	32.41	34.74	36.93	38.89	40.57	41.88	42.78	43.27	43.38	43.18	42.75	42.16	41.48	40.69	39.71	38.36	36.45
14	20.46	22.57	24.70	26.81	28.89	30.88	32.75	34.46	35.95	37.19	38.16	38.86	39.33	39.61	39.76	39.85	39.89	39.88	39.71	39.11	37.70
14.5	18.13	19.98	21.83	23.66	25.44	27.15	28.77	30.27	31.62	32.81	33.85	34.73	35.49	36.16	36.79	37.42	38.07	38.74	39.31	39.47	38.58
15	15.80	17.41	19.02	20.59	22.13	23.61	25.02	26.35	27.60	28.76	29.85	30.87	31.84	32.81	33.78	34.81	35.90	37.03	38.10	38.79	38.31

Table 64 - Radial basis functions determined by *MATLAB*® for saturates mass nitrates vs time; where nitrates amount 5 means 0.125 g/l, 10 = 0.250 g/l and 15 = 0.375g/l (Figure 3-15c)

Saturates / mg	**Nitrates**																				
Time / days	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
3	6.74	8.08	9.38	10.62	11.79	12.85	13.79	14.57	15.17	15.57	15.76	15.73	15.48	15.03	14.40	13.60	12.66	11.60	10.46	9.24	7.97
3.2	9.82	11.41	12.97	14.48	15.92	17.25	18.44	19.43	20.20	20.71	20.94	20.87	20.52	19.90	19.05	18.00	16.79	15.46	14.04	12.56	11.04
3.4	12.93	14.79	16.65	18.47	20.24	21.88	23.37	24.63	25.61	26.26	26.53	26.41	25.92	25.09	23.97	22.62	21.09	19.44	17.70	15.92	14.12
3.6	16.01	18.17	20.36	22.54	24.67	26.69	28.54	30.13	31.38	32.20	32.53	32.35	31.68	30.57	29.12	27.40	25.49	23.47	21.37	19.26	17.15
3.8	19.01	21.49	24.04	26.61	29.15	31.61	33.89	35.88	37.46	38.50	38.91	38.64	37.74	36.30	34.43	32.27	29.93	27.48	24.99	22.51	20.07
4	21.85	24.68	27.60	30.58	33.59	36.53	39.31	41.79	43.79	45.12	45.63	45.25	44.04	42.16	39.80	37.13	34.28	31.37	28.45	25.59	22.81
4.2	24.48	27.63	30.94	34.35	37.84	41.31	44.67	47.73	50.26	51.97	52.62	52.08	50.47	48.04	45.08	41.82	38.43	35.02	31.66	28.41	25.28
4.4	26.80	30.27	33.95	37.79	41.76	45.78	49.75	53.48	56.68	58.92	59.76	59.00	56.84	53.73	50.08	46.18	42.21	38.30	34.50	30.86	27.41
4.6	28.73	32.50	36.52	40.75	45.18	49.73	54.31	58.75	62.71	65.64	66.78	65.69	62.81	58.90	54.51	49.97	45.46	41.07	36.86	32.86	29.10
4.8	30.21	34.23	38.54	43.12	47.93	52.92	58.02	63.07	67.78	71.49	73.00	71.50	67.81	63.11	58.07	52.98	48.00	43.19	38.62	34.31	30.28
5	31.17	35.39	39.94	44.78	49.88	55.18	60.60	66.00	71.10	75.25	77.00	75.22	71.04	65.90	60.47	55.02	49.69	44.57	39.70	35.13	30.88
5.2	31.57	35.94	40.66	45.69	50.96	56.41	61.91	67.30	72.25	76.09	77.62	76.01	72.08	67.04	61.57	55.99	50.47	45.13	40.04	35.27	30.86
5.4	31.39	35.85	40.69	45.83	51.19	56.65	62.05	67.16	71.61	74.81	75.97	74.67	71.32	66.73	61.48	55.94	50.36	44.88	39.64	34.72	30.20
5.6	30.61	35.12	40.03	45.25	50.64	56.05	61.26	66.01	69.94	72.58	73.46	72.37	69.52	65.38	60.42	55.01	49.43	43.86	38.50	33.47	28.88
5.8	29.25	33.78	38.75	44.03	49.44	54.78	59.81	64.25	67.77	70.03	70.73	69.75	67.21	63.41	58.70	53.40	47.81	42.17	36.69	31.56	26.93
6	27.33	31.86	36.90	42.27	47.74	53.05	57.95	62.16	65.41	67.42	68.00	67.07	64.70	61.11	56.55	51.31	45.67	39.90	34.27	29.02	24.37
6.2	24.91	29.44	34.59	40.10	45.68	51.03	55.86	59.94	63.01	64.87	65.36	64.45	62.17	58.68	54.18	48.92	43.17	37.21	31.36	25.95	21.26
6.4	22.07	26.62	31.96	37.70	43.44	48.87	53.70	57.70	60.66	62.42	62.86	61.94	59.69	56.24	51.75	46.42	40.50	34.29	28.13	22.46	17.71
6.6	19.00	23.62	29.25	35.28	41.23	46.74	51.58	55.53	58.42	60.10	60.50	59.56	57.33	53.89	49.39	43.98	37.90	31.40	24.86	18.81	13.95
6.8	16.16	20.90	26.87	33.16	39.24	44.80	49.60	53.48	56.29	57.92	58.27	57.33	55.12	51.71	47.22	41.79	35.61	28.90	22.00	15.52	10.49
7	14.53	19.29	25.35	31.66	37.69	43.15	47.84	51.60	54.31	55.86	56.17	55.24	53.07	49.73	45.32	39.98	33.85	27.15	20.19	13.56	8.48

Table 65 - Radial basis functions determined by *MATLAB*® for saturates mass iron vs time; where iron amount 5 means 2.49 mg/l, 10 = 4.98 mg/l and 15 = 7.47 mg/l (Figure 3-15d)

Saturates / mg	**Iron**																				
Time / days	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
3	7.73	8.95	10.13	11.26	12.32	13.29	14.13	14.82	15.33	15.65	15.76	15.66	15.35	14.86	14.20	13.41	12.51	11.53	10.50	9.46	8.40
3.2	10.89	12.34	13.76	15.15	16.47	17.69	18.77	19.67	20.36	20.78	20.94	20.81	20.41	19.77	18.91	17.90	16.76	15.53	14.27	12.99	11.73
3.4	14.10	15.80	17.50	19.18	20.80	22.33	23.70	24.86	25.75	26.32	26.53	26.37	25.85	25.02	23.93	22.64	21.22	19.72	18.18	16.65	15.15
3.6	17.32	19.29	21.29	23.29	25.26	27.14	28.86	30.34	31.49	32.24	32.53	32.33	31.66	30.60	29.22	27.61	25.86	24.04	22.21	20.40	18.65
3.8	20.49	22.75	25.07	27.42	29.77	32.06	34.19	36.06	37.54	38.52	38.91	38.65	37.80	36.45	34.71	32.74	30.62	28.45	26.29	24.19	22.18
4	23.57	26.11	28.76	31.49	34.25	36.98	39.59	41.93	43.83	45.12	45.63	45.30	44.20	42.48	40.33	37.93	35.40	32.86	30.37	27.98	25.71
4.2	26.50	29.32	32.29	35.38	38.57	41.79	44.93	47.82	50.25	51.94	52.62	52.19	50.76	48.59	45.94	43.07	40.12	37.20	34.39	31.71	29.20
4.4	29.21	32.29	35.55	39.00	42.60	46.30	50.00	53.53	56.62	58.84	59.76	59.18	57.30	54.56	51.36	47.99	44.62	41.36	38.25	35.32	32.60
4.6	31.66	34.95	38.46	42.21	46.17	50.31	54.55	58.75	62.58	65.51	66.78	65.96	63.48	60.08	56.32	52.51	48.78	45.23	41.89	38.77	35.87
4.8	33.79	37.24	40.94	44.91	49.14	53.61	58.28	63.03	67.58	71.30	73.00	71.87	68.72	64.73	60.54	56.41	52.45	48.72	45.23	41.99	38.97
5	35.57	39.12	42.93	47.01	51.38	56.02	60.89	65.91	70.82	74.99	77.00	75.71	72.26	68.05	63.73	59.52	55.52	51.75	48.24	44.95	41.89
5.2	36.99	40.56	44.39	48.49	52.85	57.47	62.28	67.18	71.88	75.76	77.62	76.65	73.65	69.81	65.76	61.77	57.93	54.29	50.87	47.65	44.61
5.4	38.04	41.56	45.33	49.34	53.58	58.00	62.53	67.02	71.16	74.40	75.97	75.47	73.30	70.20	66.74	63.19	59.71	56.35	53.14	50.07	47.14
5.6	38.73	42.17	45.81	49.64	53.65	57.76	61.90	65.87	69.40	72.08	73.46	73.35	71.93	69.65	66.89	63.94	60.95	57.98	55.08	52.25	49.47
5.8	39.11	42.40	45.86	49.47	53.19	56.96	60.66	64.13	67.15	69.44	70.73	70.91	70.10	68.54	66.49	64.18	61.74	59.25	56.74	54.21	51.65
6	39.21	42.33	45.58	48.93	52.35	55.75	59.05	62.10	64.72	66.74	68.00	68.43	68.09	67.13	65.74	64.06	62.20	60.23	58.17	55.99	53.67
6.2	39.07	42.00	45.03	48.12	51.23	54.30	57.24	59.94	62.27	64.11	65.36	66.00	66.04	65.60	64.77	63.68	62.40	60.98	59.40	57.61	55.58
6.4	38.74	41.48	44.28	47.12	49.95	52.71	55.34	57.76	59.87	61.59	62.86	63.66	64.02	64.00	63.67	63.11	62.38	61.50	60.43	59.09	57.35
6.6	38.27	40.82	43.40	46.00	48.57	51.07	53.44	55.63	57.57	59.20	60.50	61.44	62.05	62.37	62.46	62.36	62.13	61.77	61.23	60.36	58.95
6.8	37.69	40.05	42.43	44.81	47.15	49.42	51.58	53.58	55.39	56.96	58.27	59.32	60.13	60.72	61.14	61.42	61.60	61.68	61.63	61.25	60.18
7	37.02	39.21	41.41	43.60	45.74	47.82	49.79	51.64	53.34	54.85	56.17	57.30	58.26	59.05	59.70	60.26	60.74	61.14	61.42	61.39	60.60

Table 66 - Radial basis functions determined by *MATLAB*® for saturates mass iron vs phosphates; where iron amount 5 means 2.49 mg/l, 10 = 4.98 mg/l and 15 = 7.47 mg/l; where phosphate amount 5 means a ratio of K_2HPO_4:KH_2PO_4 of 0.0375:0.0875 g/l, 10 = 0.0750:0.1750 g/l and 15 = 0.1125:0.2625 g/l (Figure 3-15e)

Saturates / mg	**Iron**																				
Phosphates	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
5	32.63	34.79	36.93	38.99	40.92	42.69	44.21	45.42	46.26	46.68	46.62	46.06	45.01	43.47	41.48	39.08	36.34	33.32	30.16	27.11	24.72
5.5	33.52	35.85	38.17	40.43	42.59	44.57	46.31	47.73	48.75	49.30	49.34	48.83	47.77	46.19	44.13	41.65	38.82	35.73	32.53	29.49	27.04
6	34.33	36.84	39.36	41.85	44.24	46.48	48.48	50.14	51.38	52.10	52.25	51.80	50.74	49.12	46.99	44.45	41.59	38.51	35.39	32.45	30.00
6.5	35.05	37.74	40.48	43.20	45.87	48.40	50.70	52.65	54.15	55.09	55.37	54.97	53.90	52.22	50.02	47.41	44.51	41.46	38.40	35.53	33.03
7	35.66	38.54	41.49	44.48	47.44	50.30	52.95	55.25	57.07	58.26	58.70	58.34	57.22	55.43	53.11	50.39	47.43	44.35	41.31	38.44	35.85
7.5	36.14	39.19	42.37	45.62	48.90	52.12	55.17	57.90	60.12	61.62	62.24	61.90	60.66	58.68	56.15	53.26	50.17	47.02	43.93	41.00	38.31
8	36.45	39.67	43.06	46.57	50.17	53.79	57.29	60.52	63.23	65.13	65.95	65.58	64.13	61.84	59.00	55.85	52.58	49.30	46.11	43.09	40.29
8.5	36.57	39.94	43.51	47.27	51.17	55.17	59.16	62.96	66.27	68.68	69.76	69.28	67.45	64.70	61.45	57.98	54.47	51.03	47.74	44.62	41.70
9	36.48	39.96	43.67	47.62	51.78	56.12	60.56	64.93	68.94	72.02	73.42	72.71	70.31	66.98	63.25	59.44	55.69	52.09	48.68	45.48	42.48
9.5	36.15	39.69	43.49	47.55	51.88	56.45	61.22	66.06	70.71	74.52	76.30	75.25	72.17	68.24	64.09	60.00	56.08	52.36	48.87	45.61	42.56
10	35.57	39.12	42.93	47.01	51.38	56.02	60.89	65.91	70.82	74.99	77.00	75.71	72.26	68.05	63.73	59.52	55.52	51.75	48.24	44.95	41.89
10.5	34.74	38.23	41.98	45.99	50.26	54.78	59.49	64.29	68.88	72.65	74.39	73.29	70.18	66.21	62.04	57.92	53.98	50.25	46.76	43.50	40.48
11	33.67	37.05	40.66	44.50	48.56	52.79	57.12	61.40	65.31	68.29	69.60	68.81	66.34	62.93	59.15	55.28	51.50	47.88	44.47	41.28	38.33
11.5	32.38	35.60	39.02	42.62	46.37	50.21	54.04	57.69	60.85	63.13	64.07	63.47	61.53	58.68	55.33	51.79	48.22	44.74	41.44	38.34	35.50
12	30.90	33.93	37.11	40.42	43.81	47.22	50.53	53.56	56.07	57.79	58.44	57.91	56.30	53.87	50.91	47.66	44.29	40.96	37.76	34.77	32.04
12.5	29.26	32.08	35.00	38.00	41.03	44.00	46.81	49.29	51.27	52.55	52.95	52.42	50.99	48.84	46.15	43.12	39.92	36.68	33.56	30.65	28.05
13	27.50	30.09	32.76	35.45	38.12	40.68	43.04	45.06	46.61	47.53	47.72	47.13	45.79	43.79	41.28	38.39	35.27	32.08	28.97	26.11	23.62
13.5	25.65	28.02	30.42	32.82	35.15	37.35	39.32	40.95	42.14	42.78	42.78	42.12	40.79	38.88	36.45	33.64	30.55	27.34	24.17	21.28	18.89
14	23.73	25.89	28.05	30.17	32.20	34.07	35.71	37.02	37.92	38.32	38.16	37.41	36.07	34.19	31.82	29.03	25.94	22.66	19.38	16.38	14.02
14.5	21.79	23.74	25.67	27.54	29.30	30.89	32.24	33.28	33.94	34.14	33.85	33.02	31.67	29.81	27.47	24.73	21.64	18.31	14.91	11.74	9.37
15	19.82	21.58	23.30	24.95	26.47	27.81	28.93	29.74	30.20	30.25	29.85	28.97	27.60	25.77	23.50	20.83	17.82	14.54	11.15	7.96	5.63

Table 67 - Radial basis functions determined by *MATLAB*® for saturates mass phosphates vs time; where phosphate amount 5 means a ratio of K_2HPO_4:KH_2PO_4 of 0.0375:0.0875 g/l, 10 = 0.0750:0.1750 g/l and 15 = 0.1125:0.2625 g/l (Figure 3-15f)

Saturates / mg	**Phosphates**																				
Time / days	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
3	0.07	1.90	4.11	6.41	8.65	10.72	12.52	13.99	15.05	15.66	15.76	15.35	14.41	12.97	11.07	8.76	6.10	3.17	0.09	-2.89	-5.31
3.2	3.73	5.70	8.05	10.53	12.97	15.24	17.25	18.90	20.10	20.80	20.94	20.49	19.46	17.88	15.80	13.28	10.41	7.29	4.05	0.95	-1.62
3.4	7.89	10.00	12.44	15.05	17.66	20.14	22.35	24.19	25.56	26.36	26.53	26.04	24.90	23.15	20.87	18.16	15.10	11.84	8.52	5.38	2.68
3.6	12.33	14.58	17.15	19.89	22.67	25.36	27.80	29.86	31.41	32.33	32.53	31.98	30.69	28.74	26.24	23.29	20.04	16.63	13.22	9.99	7.12
3.8	16.92	19.33	22.04	24.94	27.92	30.83	33.53	35.84	37.61	38.67	38.91	38.27	36.79	34.57	31.78	28.56	25.07	21.47	17.90	14.53	11.46
4	21.62	24.19	27.06	30.13	33.31	36.47	39.46	42.08	44.11	45.36	45.63	44.85	43.10	40.54	37.37	33.80	30.02	26.17	22.41	18.84	15.55
4.2	26.42	29.13	32.13	35.37	38.75	42.17	45.47	48.44	50.82	52.30	52.62	51.65	49.52	46.48	42.84	38.85	34.72	30.60	26.61	22.83	19.32
4.4	31.32	34.13	37.23	40.58	44.13	47.77	51.38	54.74	57.54	59.37	59.76	58.51	55.84	52.18	47.96	43.51	39.00	34.60	30.37	26.39	22.69
4.6	36.31	39.16	42.30	45.70	49.32	53.10	56.95	60.68	63.97	66.25	66.78	65.12	61.71	57.31	52.48	47.55	42.70	38.03	33.60	29.45	25.59
4.8	41.40	44.23	47.31	50.64	54.21	57.98	61.89	65.83	69.51	72.30	73.00	70.82	66.56	61.43	56.06	50.76	45.64	40.78	36.21	31.95	27.99
5	46.62	49.34	52.25	55.37	58.70	62.24	65.95	69.76	73.42	76.30	77.00	74.39	69.60	64.07	58.44	52.95	47.72	42.78	38.16	33.85	29.85
5.2	51.96	54.49	57.12	59.87	62.77	65.82	69.00	72.23	75.24	77.43	77.62	75.00	70.41	65.02	59.48	54.07	48.89	44.00	39.42	35.15	31.17
5.4	57.46	59.72	61.94	64.17	66.44	68.76	71.13	73.43	75.38	76.47	75.97	73.47	69.39	64.48	59.31	54.16	49.19	44.47	40.03	35.86	31.97
5.6	63.13	65.03	66.74	68.30	69.78	71.20	72.56	73.76	74.57	74.60	73.46	70.95	67.29	62.89	58.18	53.41	48.75	44.28	40.04	36.05	32.30
5.8	68.98	70.47	71.56	72.32	72.87	73.27	73.54	73.61	73.32	72.43	70.73	68.10	64.67	60.68	56.39	52.02	47.70	43.53	39.55	35.78	32.21
6	75.02	76.05	76.41	76.26	75.77	75.08	74.23	73.21	71.92	70.22	68.00	65.19	61.86	58.14	54.20	50.20	46.23	42.37	38.66	35.13	31.78
6.2	81.22	81.73	81.26	80.08	78.48	76.65	74.70	72.66	70.47	68.06	65.36	62.34	59.02	55.48	51.82	48.12	44.47	40.91	37.49	34.20	31.07
6.4	87.49	87.42	85.98	83.66	80.89	77.94	74.96	71.99	69.01	65.98	62.86	59.62	56.27	52.84	49.38	45.94	42.56	39.28	36.12	33.08	30.17
6.6	93.57	92.83	90.27	86.73	82.81	78.83	74.94	71.17	67.53	63.98	60.50	57.05	53.65	50.29	46.98	43.75	40.61	37.58	34.65	31.84	29.15
6.8	98.74	97.27	93.53	88.86	83.97	79.16	74.54	70.16	65.99	62.04	58.27	54.66	51.21	47.89	44.70	41.64	38.69	35.87	33.16	30.55	28.05
7	101.41	99.40	94.91	89.58	84.11	78.78	73.70	68.89	64.38	60.14	56.17	52.45	48.96	45.68	42.59	39.66	36.88	34.23	31.70	29.27	26.94

Table 68 - Error graphs for saturated FAME content derived from algal cells: (1) percentage and (2) mass produced (Nitrates – $NaNO_3$, phosphates – K_2HPO_4:KH_2PO_4, iron – $FeSO_4.7H_2O$, time – days after inoculation) with key to error (red high error, blue low error) (Figure 3-14, Figure 3-15)

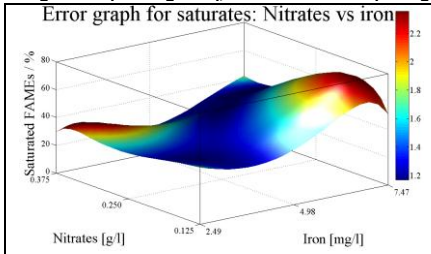
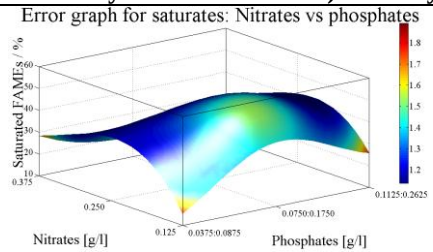
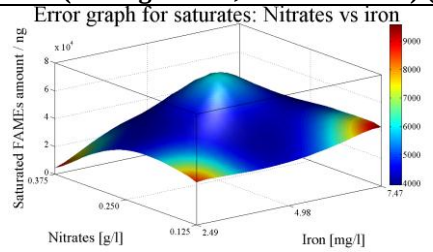
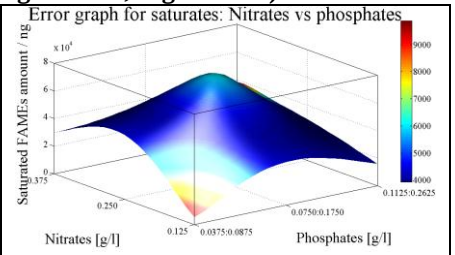
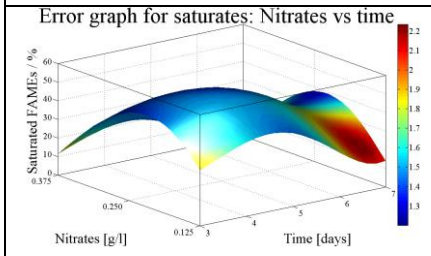
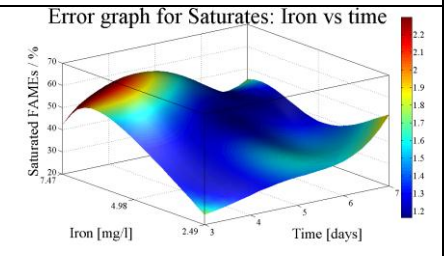
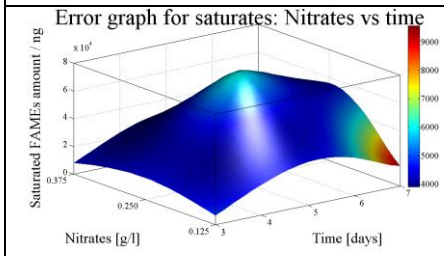
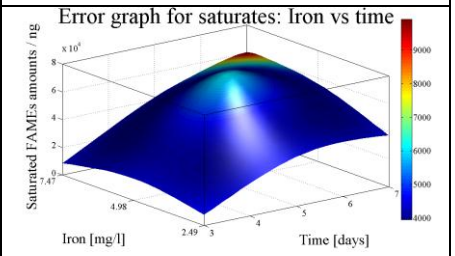
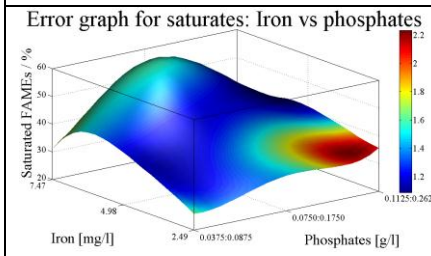
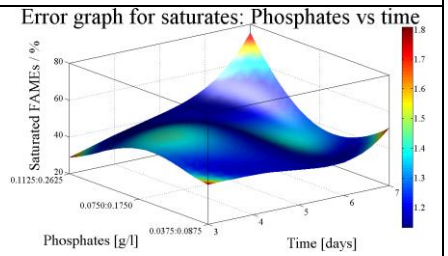
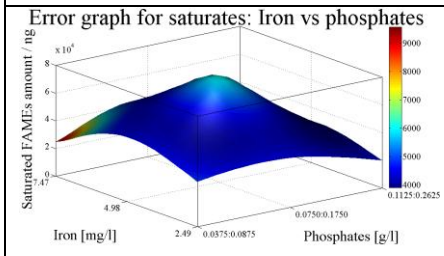
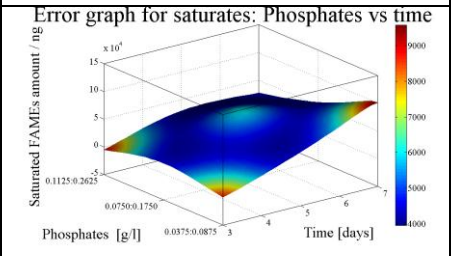
 **1a**	 **1b**	 **2a**	 **2b**
 **1c**	 **1d**	 **2c**	 **2d**
 **1e**	 **1f**	 **2e**	 **2f**

Table 69 - Radial basis functions determined by *MATLAB*® for total FAMEs mass nitrates vs iron; where nitrates amount 5 means 0.125 g/l, 10 = 0.250 g/l and 15 = 0.375g/l; where iron amount 5 means 2.49 mg/l, 10 = 4.98 mg/l and 15 = 7.47 mg/l (Figure 3-16a)

Total / mg	**Nitrates**																				
Iron	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
5	98.94	101.55	102.43	102.09	100.98	99.36	97.33	94.89	91.99	88.53	84.45	79.68	74.17	67.95	61.05	53.57	45.70	37.72	30.06	23.35	18.23
5.5	99.60	102.89	104.60	105.18	105.03	104.38	103.29	101.73	99.61	96.83	93.27	88.86	83.59	77.46	70.55	62.99	54.98	46.83	38.98	32.03	26.58
6	98.55	102.63	105.41	107.22	108.33	108.93	109.05	108.61	107.49	105.55	102.65	98.72	93.75	87.78	80.93	73.38	65.36	57.20	49.33	42.29	36.55
6.5	96.40	101.32	105.28	108.46	111.04	113.11	114.65	115.54	115.60	114.65	112.52	109.13	104.50	98.71	91.94	84.42	76.43	68.32	60.49	53.36	47.33
7	93.72	99.49	104.63	109.23	113.35	117.02	120.12	122.50	123.89	124.04	122.76	119.93	115.61	109.95	103.21	95.68	87.69	79.59	71.74	64.49	58.13
7.5	90.92	97.50	103.78	109.75	115.41	120.70	125.45	129.40	132.23	133.58	133.17	130.88	126.79	121.16	114.32	106.68	98.59	90.41	82.46	75.03	68.31
8	88.28	95.61	102.91	110.14	117.26	124.12	130.50	136.08	140.40	142.99	143.45	141.61	137.62	131.85	124.78	116.89	108.60	100.24	92.11	84.44	77.37
8.5	85.96	93.93	102.12	110.46	118.86	127.15	135.07	142.21	148.01	151.84	153.12	151.61	147.54	141.46	134.02	125.77	117.19	108.59	100.24	92.30	84.91
9	84.05	92.54	101.43	110.66	120.10	129.58	138.80	147.32	154.47	159.43	161.43	160.12	155.82	149.32	141.42	132.78	123.88	115.03	106.44	98.27	90.60
9.5	82.61	91.46	100.84	110.67	120.84	131.15	141.30	150.82	158.99	164.83	167.35	166.13	161.57	154.67	146.39	137.43	128.27	119.21	110.44	102.08	94.22
10	81.67	90.71	100.33	110.45	120.96	131.66	142.24	152.20	160.82	167.05	169.82	168.64	163.98	156.92	148.47	139.36	130.07	120.89	112.02	103.57	95.61
10.5	81.24	90.27	99.87	109.96	120.42	131.02	141.46	151.24	159.64	165.67	168.34	167.20	162.70	155.80	147.47	138.44	129.18	120.01	111.13	102.66	94.70
11	81.29	90.13	99.47	109.22	119.24	129.31	139.10	148.15	155.76	161.11	163.39	162.27	158.06	151.55	143.57	134.79	125.69	116.61	107.80	99.41	91.55
11.5	81.78	90.28	99.15	108.28	117.54	126.72	135.50	143.44	149.94	154.33	156.04	154.81	150.88	144.79	137.21	128.74	119.86	110.93	102.22	93.96	86.29
12	82.65	90.68	98.89	107.19	115.47	123.52	131.06	137.70	142.94	146.29	147.31	145.84	142.02	136.23	128.98	120.79	112.09	103.28	94.68	86.57	79.13
12.5	83.82	91.27	98.68	106.01	113.14	119.92	126.12	131.40	135.37	137.66	137.94	136.10	132.21	126.55	119.49	111.45	102.85	94.09	85.55	77.57	70.39
13	85.15	91.95	98.48	104.71	110.62	116.09	120.92	124.87	127.62	128.87	128.40	126.10	122.01	116.31	109.29	101.28	92.67	83.86	75.30	67.39	60.48
13.5	86.44	92.55	98.13	103.25	107.91	112.07	115.60	118.29	119.90	120.20	118.99	116.19	111.81	105.97	98.87	90.80	82.09	73.15	64.47	56.58	49.92
14	87.40	92.81	97.45	101.46	104.93	107.88	110.21	111.77	112.36	111.78	109.90	106.62	101.92	95.89	88.68	80.50	71.67	62.58	53.76	45.83	39.40
14.5	87.66	92.39	96.15	99.15	101.57	103.45	104.74	105.33	105.04	103.72	101.24	97.53	92.56	86.36	79.07	70.84	61.95	52.79	43.90	36.00	29.80
15	86.80	90.93	93.96	96.16	97.72	98.74	99.19	98.97	97.97	96.05	93.09	89.04	83.86	77.59	70.29	62.14	53.37	44.34	35.60	27.90	22.00

Table 70 - Radial basis functions determined by *MATLAB*® for total FAMEs mass nitrates vs phosphates; where nitrates amount 5 means 0.125 g/l, 10 = 0.250 g/l and 15 = 0.375g/l; where phosphates of K_2HPO_4:KH_2PO_4 of 0.0375:0.0875 g/l, 10 = 0.0750:0.1750 g/l and 15 = 0.1125:0.2625 g/l (Figure 3-16b)

Total / mg	**Nitrates**																				
Phosphates	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
5	19.22	28.57	40.20	53.19	66.68	79.99	92.67	104.39	114.93	124.17	132.04	138.57	143.83	147.95	151.10	153.41	154.96	155.72	155.44	153.64	149.78
5.5	24.30	33.82	45.52	58.54	72.04	85.35	98.00	109.62	119.97	128.90	136.32	142.26	146.79	150.09	152.32	153.67	154.25	154.06	152.89	150.31	145.87
6	31.46	41.06	52.58	65.34	78.58	91.66	104.08	115.45	125.48	133.96	140.79	145.98	149.60	151.84	152.92	153.04	152.35	150.89	148.52	144.93	139.81
6.5	39.82	49.44	60.70	73.07	85.91	98.63	110.72	121.76	131.39	139.35	145.49	149.79	152.34	153.35	153.06	151.74	149.58	146.67	142.95	138.28	132.46
7	48.49	58.14	69.16	81.14	93.56	105.90	117.65	128.35	137.58	145.00	150.41	153.74	155.10	154.73	152.94	150.06	146.33	141.92	136.87	131.13	124.66
7.5	56.83	66.50	77.32	88.97	101.03	113.04	124.52	134.95	143.86	150.81	155.50	157.83	157.91	156.08	152.72	148.24	142.96	137.11	130.81	124.12	117.05
8	64.38	74.05	84.70	96.08	107.86	119.63	130.92	141.21	149.94	156.54	160.59	161.92	160.72	157.39	152.47	146.45	139.72	132.58	125.20	117.70	110.14
8.5	70.81	80.45	90.93	102.08	113.61	125.20	136.39	146.67	155.37	161.78	165.32	165.74	163.27	158.51	152.13	144.73	136.77	128.56	120.33	112.21	104.29
9	75.92	85.45	95.73	106.62	117.91	129.31	140.43	150.74	159.51	165.89	169.07	168.70	165.12	159.11	151.53	143.06	134.18	125.22	116.42	107.91	99.79
9.5	79.56	88.90	98.90	109.46	120.42	131.55	142.51	152.77	161.58	167.96	170.91	169.97	165.58	158.73	150.39	141.30	131.94	122.65	113.61	104.99	96.84
10	81.67	90.71	100.33	110.45	120.96	131.66	142.24	152.20	160.82	167.05	169.82	168.64	163.98	156.92	148.47	139.36	130.07	120.89	112.02	103.57	95.61
10.5	82.25	90.88	100.00	109.57	119.47	129.54	139.48	148.84	156.92	162.75	165.36	164.32	160.05	153.52	145.67	137.21	128.56	120.01	111.70	103.74	96.16
11	81.38	89.50	98.03	106.92	116.07	125.33	134.42	142.93	150.22	155.51	158.03	157.45	154.11	148.75	142.15	134.94	127.49	120.03	112.69	105.50	98.50
11.5	79.20	86.72	94.57	102.69	111.00	119.35	127.49	135.05	141.52	146.30	148.86	148.98	146.88	143.09	138.21	132.71	126.91	120.97	114.94	108.82	102.57
12	75.87	82.74	89.86	97.18	104.62	112.04	119.23	125.90	131.66	136.08	138.83	139.78	139.07	137.06	134.15	130.68	126.87	122.79	118.38	113.55	108.20
12.5	71.61	77.81	84.19	90.70	97.28	103.82	110.15	116.06	121.28	125.54	128.62	130.48	131.20	131.01	130.19	128.93	127.33	125.35	122.80	119.46	115.13
13	66.63	72.16	77.82	83.57	89.35	95.10	100.69	106.00	110.86	115.10	118.62	121.40	123.53	125.13	126.38	127.38	128.09	128.37	127.87	126.17	122.93
13.5	61.15	66.05	71.02	76.06	81.14	86.21	91.20	96.06	100.69	105.03	109.03	112.72	116.16	119.44	122.65	125.81	128.82	131.39	133.02	133.07	130.95
14	55.36	59.67	64.03	68.45	72.91	77.41	81.94	86.46	90.97	95.47	99.95	104.47	109.07	113.83	118.79	123.91	129.02	133.74	137.45	139.23	138.24
14.5	49.45	53.23	57.05	60.93	64.88	68.92	73.08	77.37	81.83	86.49	91.40	96.62	102.20	108.18	114.56	121.27	128.11	134.64	140.11	143.42	143.43
15	43.56	46.87	50.23	53.66	57.20	60.88	64.75	68.87	73.31	78.13	83.39	89.15	95.46	102.33	109.75	117.59	125.62	133.36	140.00	144.35	145.16

Table 71 - Radial basis functions determined by *MATLAB*® for total FAMEs mass nitrates vs time; where nitrates amount 5 means 0.125 g/l, 10 = 0.250 g/l and 15 = 0.375g/l (Figure 3-16c)

Total / mg	**Nitrates**																				
Time / days	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
3	15.36	18.11	20.85	23.54	26.17	28.72	31.15	33.44	35.55	37.47	39.15	40.59	41.77	42.69	43.33	43.71	43.82	43.66	43.24	42.57	41.66
3.2	23.32	26.64	29.96	33.27	36.51	39.64	42.62	45.38	47.87	50.03	51.81	53.20	54.18	54.76	54.97	54.84	54.38	53.64	52.62	51.35	49.85
3.4	31.40	35.36	39.35	43.35	47.29	51.12	54.75	58.08	61.02	63.47	65.36	66.66	67.37	67.52	67.18	66.40	65.27	63.82	62.11	60.16	58.00
3.6	39.49	44.13	48.86	53.63	58.37	63.00	67.39	71.39	74.87	77.67	79.68	80.85	81.20	80.80	79.77	78.23	76.28	74.02	71.50	68.79	65.91
3.8	47.42	52.78	58.30	63.91	69.53	75.06	80.31	85.11	89.22	92.43	94.57	95.56	95.45	94.37	92.51	90.05	87.17	83.98	80.58	77.04	73.39
4	55.02	61.12	67.45	73.95	80.51	87.01	93.24	98.94	103.80	107.49	109.77	110.52	109.84	107.92	105.07	101.56	97.62	93.42	89.07	84.65	80.19
4.2	62.11	68.94	76.08	83.47	91.00	98.52	105.80	112.50	118.21	122.46	124.89	125.35	123.94	121.03	117.04	112.36	107.28	102.00	96.66	91.34	86.07
4.4	68.49	76.01	83.92	92.17	100.64	109.18	117.52	125.27	131.90	136.79	139.39	139.46	137.21	133.17	127.93	122.00	115.74	109.37	103.04	96.83	90.78
4.6	73.98	82.12	90.72	99.74	109.06	118.53	127.85	136.61	144.16	149.69	152.44	152.07	148.90	143.67	137.17	130.01	122.59	115.17	107.90	100.85	94.07
4.8	78.42	87.07	96.25	105.90	115.92	126.14	136.25	145.79	154.07	160.12	162.96	162.15	158.10	151.80	144.17	135.91	127.46	119.09	110.96	103.15	95.73
5	81.67	90.71	100.33	110.45	120.96	131.66	142.24	152.20	160.82	167.05	169.82	168.64	163.98	156.92	148.47	139.36	130.07	120.89	112.02	103.57	95.61
5.2	83.65	92.95	102.85	113.27	124.05	134.94	145.62	155.56	164.02	170.00	172.50	171.07	166.16	158.76	149.85	140.19	130.28	120.46	110.98	101.99	93.60
5.4	84.34	93.76	103.81	114.35	125.19	136.04	146.50	156.05	163.96	169.36	171.44	169.86	164.96	157.53	148.47	138.49	128.13	117.81	107.83	98.41	89.70
5.6	83.74	93.17	103.25	113.81	124.57	135.20	145.26	154.22	161.41	166.11	167.71	165.97	161.14	153.81	144.72	134.54	123.84	113.10	102.69	92.91	83.98
5.8	81.93	91.29	101.33	111.82	122.43	132.77	142.37	150.70	157.16	161.19	162.32	160.38	155.55	148.27	139.14	128.77	117.75	106.59	95.76	85.67	76.59
6	79.06	88.28	98.23	108.63	119.08	129.14	138.30	146.06	151.89	155.32	156.01	153.82	148.89	141.55	132.30	121.68	110.27	98.65	87.38	76.96	67.79
6.2	75.33	84.36	94.23	104.56	114.88	124.69	133.48	140.75	146.05	148.98	149.26	146.80	141.69	134.19	124.72	113.77	101.91	89.76	77.97	67.20	57.96
6.4	71.05	79.87	89.66	99.95	110.17	119.77	128.25	135.12	139.98	142.48	142.40	139.66	134.32	126.61	116.87	105.55	93.20	80.47	68.12	56.94	47.64
6.6	66.66	75.25	84.97	95.20	105.32	114.72	122.90	129.42	133.89	136.03	135.63	132.63	127.08	119.17	109.18	97.53	84.74	71.48	58.58	47.00	37.67
6.8	62.76	71.09	80.65	90.76	100.68	109.82	117.68	123.83	127.95	129.76	129.09	125.88	120.18	112.13	102.01	90.18	77.13	63.54	50.26	38.42	29.11
7	59.99	67.97	77.21	86.99	96.55	105.30	112.74	118.50	122.25	123.76	122.87	119.52	113.75	105.70	95.62	83.84	70.85	57.28	44.03	32.24	23.11

Table 72 - Radial basis functions determined by *MATLAB*® for total FAMEs mass iron vs time; where iron amount 5 means 2.49 mg/l, 10 = 4.98 mg/l and 15 = 7.47 mg/l (Figure 3-16d)

Total / mg	Iron																				
Time / days	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
3	22.12	25.01	27.80	30.44	32.86	34.99	36.76	38.11	38.98	39.34	39.15	38.42	37.17	35.43	33.27	30.75	27.95	24.97	21.87	18.73	15.61
3.2	29.54	33.05	36.51	39.83	42.93	45.72	48.11	49.99	51.28	51.90	51.81	51.00	49.51	47.38	44.71	41.60	38.17	34.53	30.78	27.02	23.32
3.4	37.04	41.23	45.41	49.49	53.38	56.94	60.05	62.57	64.36	65.32	65.36	64.47	62.68	60.09	56.83	53.04	48.88	44.50	40.03	35.58	31.26
3.6	44.49	49.41	54.37	59.29	64.05	68.48	72.43	75.71	78.11	79.47	79.68	78.69	76.57	73.44	69.49	64.92	59.95	54.76	49.51	44.34	39.35
3.8	51.79	57.44	63.22	69.03	74.73	80.13	85.03	89.17	92.30	94.15	94.57	93.48	90.97	87.22	82.49	77.07	71.21	65.16	59.11	53.19	47.52
4	58.77	65.16	71.77	78.49	85.16	91.60	97.55	102.68	106.65	109.10	109.77	108.57	105.60	101.15	95.56	89.22	82.45	75.52	68.65	62.00	55.68
4.2	65.31	72.40	79.80	87.41	95.07	102.57	109.62	115.83	120.76	123.91	124.89	123.57	120.09	114.86	108.36	101.08	93.40	85.62	77.99	70.65	63.72
4.4	71.26	78.98	87.09	95.52	104.10	112.62	120.77	128.12	134.09	138.03	139.39	137.94	133.90	127.85	120.45	112.27	103.76	95.24	86.93	79.01	71.56
4.6	76.50	84.73	93.44	102.55	111.92	121.33	130.48	138.88	145.89	150.67	152.44	150.88	146.30	139.50	131.31	122.39	113.23	104.13	95.33	86.96	79.11
4.8	80.92	89.53	98.67	108.27	118.21	128.29	138.18	147.42	155.28	160.79	162.96	161.35	156.41	149.10	140.41	131.05	121.51	112.10	103.02	94.39	86.31
5	84.45	93.27	102.65	112.52	122.76	133.17	143.45	153.12	161.43	167.35	169.82	168.34	163.39	156.04	147.31	137.94	128.40	118.99	109.90	101.24	93.09
5.2	87.06	95.91	105.32	115.21	125.45	135.85	146.09	155.70	163.96	169.88	172.50	171.35	166.87	160.05	151.83	142.92	133.80	124.74	115.93	107.47	99.45
5.4	88.74	97.47	106.70	116.38	126.35	136.41	146.24	155.39	163.18	168.79	171.44	170.79	167.16	161.32	154.07	146.06	137.73	129.35	121.10	113.08	105.35
5.6	89.55	98.00	106.90	116.16	125.63	135.11	144.29	152.73	159.87	165.05	167.71	167.62	164.99	160.38	154.40	147.59	140.34	132.91	125.46	118.08	110.82
5.8	89.56	97.62	106.04	114.75	123.58	132.34	140.74	148.39	154.85	159.62	162.32	162.79	161.18	157.84	153.25	147.81	141.84	135.57	129.10	122.52	115.85
6	88.88	96.45	104.32	112.38	120.50	128.48	136.07	142.96	148.80	153.23	156.01	157.02	156.35	154.25	151.04	147.02	142.43	137.43	132.09	126.42	120.45
6.2	87.61	94.65	101.91	109.30	116.68	123.88	130.71	136.90	142.21	146.39	149.26	150.77	150.96	149.99	148.10	145.47	142.28	138.60	134.46	129.80	124.58
6.4	85.88	92.37	99.01	105.72	112.38	118.86	124.98	130.57	135.44	139.42	142.40	144.35	145.29	145.34	144.63	143.31	141.46	139.11	136.19	132.57	128.14
6.6	83.81	89.75	95.78	101.85	107.84	113.65	119.15	124.21	128.71	132.54	135.63	137.96	139.54	140.46	140.79	140.63	140.00	138.89	137.15	134.56	130.90
6.8	81.51	86.92	92.38	97.84	103.22	108.44	113.40	118.02	122.21	125.92	129.09	131.72	133.83	135.46	136.65	137.45	137.87	137.81	137.11	135.46	132.53
7	79.07	83.99	88.93	93.85	98.69	103.39	107.88	112.12	116.05	119.64	122.87	125.73	128.24	130.41	132.26	133.81	135.01	135.77	135.87	134.96	132.66

Table 73 - Radial basis functions determined by *MATLAB*® for total FAMEs mass iron vs phosphates; where iron amount 5 means 2.49 mg/l, 10 = 4.98 mg/l and 15 = 7.47 mg/l; where phosphate amount 5 means a ratio of K_2HPO_4:KH_2PO_4 of 0.0375:0.0875 g/l, 10 = 0.0750:0.1750 g/l and 15 = 0.1125:0.2625 g/l (Figure 3-16e)

Total / mg	**Iron**																				
Phosphates	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
5	109.76	114.28	118.21	121.65	124.67	127.28	129.44	131.07	132.09	132.44	132.04	130.89	129.00	126.42	123.23	119.52	115.40	110.97	106.32	101.53	96.69
5.5	109.91	114.84	119.25	123.20	126.74	129.87	132.52	134.59	135.98	136.58	136.32	135.18	133.18	130.38	126.89	122.82	118.31	113.49	108.45	103.30	98.11
6	109.29	114.69	119.67	124.24	128.44	132.22	135.48	138.11	139.95	140.88	140.79	139.67	137.53	134.46	130.61	126.13	121.17	115.90	110.43	104.88	99.32
6.5	108.04	113.94	119.54	124.82	129.77	134.31	138.32	141.62	144.02	145.36	145.49	144.38	142.06	138.66	134.37	129.39	123.93	118.15	112.20	106.21	100.27
7	106.26	112.68	118.92	124.96	130.74	136.14	141.00	145.10	148.19	150.02	150.41	149.30	146.74	142.93	138.11	132.54	126.49	120.15	113.69	107.24	100.89
7.5	104.00	110.93	117.83	124.64	131.29	137.63	143.45	148.48	152.38	154.81	155.50	154.36	151.49	147.16	141.69	135.45	128.73	121.79	114.79	107.86	101.11
8	101.24	108.67	116.21	123.79	131.33	138.65	145.52	151.58	156.42	159.54	160.59	159.39	156.12	151.13	144.92	137.92	130.50	122.92	115.37	107.98	100.83
8.5	97.97	105.85	113.98	122.30	130.69	138.99	146.93	154.10	159.96	163.89	165.32	164.03	160.25	154.53	147.50	139.71	131.58	123.38	115.31	107.48	99.98
9	94.11	102.39	111.04	120.01	129.18	138.37	147.31	155.56	162.47	167.23	169.07	167.66	163.33	156.85	149.03	140.51	131.73	122.98	114.45	106.25	98.45
9.5	89.62	98.21	107.29	116.78	126.58	136.51	146.27	155.40	163.19	168.69	170.91	169.41	164.62	157.53	149.09	140.00	130.72	121.56	112.68	104.20	96.17
10	84.45	93.27	102.65	112.52	122.76	133.17	143.45	153.12	161.43	167.35	169.82	168.34	163.39	156.04	147.31	137.94	128.40	118.99	109.90	101.24	93.09
10.5	78.59	87.52	97.08	107.17	117.65	128.28	138.74	148.52	156.87	162.82	165.36	164.06	159.33	152.18	143.58	134.25	124.70	115.23	106.08	97.36	89.18
11	72.05	81.00	90.62	100.79	111.33	121.95	132.29	141.84	149.87	155.54	158.03	157.00	152.79	146.19	138.04	129.03	119.68	110.33	101.23	92.57	84.45
11.5	64.86	73.75	83.35	93.50	103.97	114.44	124.50	133.63	141.18	146.46	148.86	148.14	144.53	138.61	131.07	122.55	113.52	104.40	95.46	86.92	78.95
12	57.11	65.87	75.40	85.48	95.84	106.09	115.81	124.50	131.59	136.51	138.83	138.38	135.31	130.05	123.14	115.13	106.48	97.63	88.89	80.53	72.75
12.5	48.91	57.51	66.95	76.96	87.20	97.26	106.68	114.99	121.70	126.35	128.62	128.39	125.76	121.05	114.68	107.13	98.84	90.24	81.69	73.53	65.98
13	40.44	48.86	58.22	68.19	78.36	88.27	97.46	105.49	111.91	116.37	118.62	118.56	116.27	111.98	106.05	98.87	90.87	82.48	74.10	66.11	58.81
13.5	31.95	40.18	49.49	59.47	69.61	79.42	88.44	96.24	102.46	106.80	109.03	109.10	107.06	103.10	97.51	90.63	82.87	74.63	66.37	58.52	51.46
14	23.82	31.87	41.14	51.12	61.23	70.95	79.81	87.44	93.50	97.72	99.95	100.12	98.28	94.58	89.26	82.65	75.08	66.98	58.82	51.10	44.29

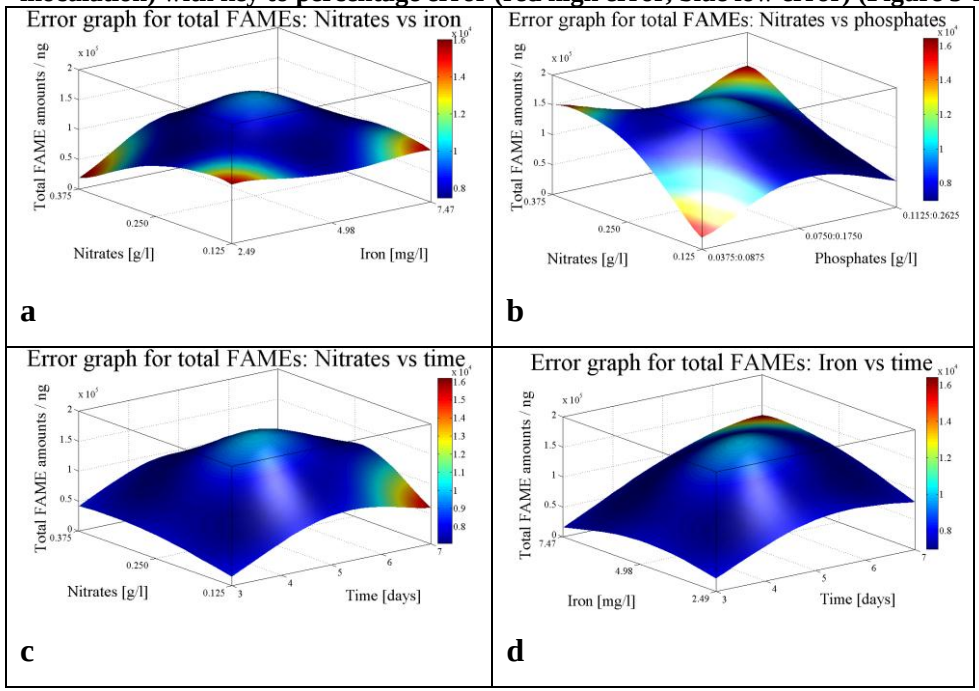
14.5	16.57	24.40	33.59	43.50	53.51	63.07	71.75	79.18	85.07	89.19	91.40	91.65	89.98	86.51	81.46	75.10	67.77	59.86	51.85	44.29	37.73
15	10.74	18.30	27.24	36.91	46.66	55.94	64.34	71.52	77.21	81.21	83.39	83.70	82.18	78.95	74.18	68.14	61.13	53.52	45.79	38.52	32.27

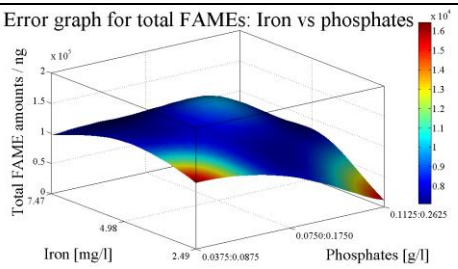
Table 74 - Radial basis functions determined by *MATLAB*® for total FAMEs mass phosphates vs time; where phosphate amount 5 means a ratio of K_2HPO_4:KH_2PO_4 of 0.0375:0.0875 g/l, 10 = 0.0750:0.1750 g/l and 15 = 0.1125:0.2625 g/l (Figure 3-16f)

Total / mg	**Phosphates**																				
Time / days	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
3	-0.85	2.27	6.79	12.12	17.71	23.11	28.03	32.23	35.55	37.88	39.15	39.34	38.46	36.57	33.78	30.22	26.06	21.50	16.82	12.36	8.46
3.2	10.26	13.67	18.42	23.98	29.78	35.41	40.53	44.89	48.31	50.64	51.81	51.78	50.58	48.30	45.04	40.98	36.31	31.24	26.07	21.14	16.75
3.4	23.12	26.77	31.63	37.24	43.12	48.85	54.09	58.56	62.04	64.34	65.36	65.07	63.49	60.74	56.97	52.37	47.18	41.63	36.01	30.63	25.75
3.6	37.12	41.01	45.92	51.53	57.41	63.19	68.52	73.07	76.58	78.83	79.68	79.06	77.03	73.73	69.36	64.18	58.43	52.37	46.30	40.46	35.08
3.8	51.68	55.80	60.78	66.38	72.26	78.09	83.50	88.16	91.73	93.93	94.57	93.56	90.98	87.03	81.97	76.11	69.73	63.13	56.55	50.23	44.33
4	66.34	70.67	75.73	81.35	87.26	93.16	98.69	103.50	107.18	109.36	109.77	108.30	105.07	100.34	94.46	87.83	80.76	73.54	66.43	59.59	53.18
4.2	80.75	85.26	90.40	96.04	101.98	107.96	113.65	118.66	122.51	124.71	124.89	122.90	118.88	113.23	106.43	98.93	91.11	83.26	75.59	68.25	61.35
4.4	94.68	99.31	104.47	110.08	116.01	122.04	127.87	133.09	137.16	139.44	139.39	136.79	131.88	125.20	117.39	109.00	100.43	91.95	83.75	75.95	68.61
4.6	107.95	112.59	117.67	123.16	128.96	134.93	140.79	146.15	150.39	152.74	152.44	149.19	143.32	135.59	126.80	117.57	108.32	99.29	90.65	82.47	74.78
4.8	120.43	124.96	129.82	135.02	140.51	146.19	151.85	157.10	161.31	163.57	162.96	159.06	152.31	143.68	134.09	124.22	114.46	105.05	96.09	87.66	79.73
5	132.04	136.32	140.79	145.49	150.41	155.50	160.59	165.32	169.07	170.91	169.82	165.36	158.03	148.86	138.83	128.62	118.62	109.03	99.95	91.40	83.39
5.2	142.78	146.65	150.54	154.50	158.57	162.72	166.81	170.53	173.29	174.25	172.50	167.63	160.11	150.87	140.85	130.67	120.72	111.20	102.18	93.69	85.71
5.4	152.65	155.96	159.09	162.09	165.03	167.91	170.63	172.92	174.28	174.01	171.44	166.28	158.86	149.95	140.29	130.47	120.84	111.60	102.83	94.56	86.76
5.6	161.71	164.34	166.54	168.38	169.96	171.32	172.41	173.02	172.76	171.15	167.71	162.27	155.07	146.64	137.57	128.32	119.21	110.42	102.05	94.13	86.63
5.8	170.01	171.86	172.99	173.51	173.57	173.26	172.59	171.44	169.55	166.61	162.32	156.59	149.58	141.65	133.21	124.61	116.13	107.91	100.05	92.57	85.46
6	177.59	178.60	178.55	177.64	176.08	174.03	171.59	168.71	165.28	161.10	156.01	149.97	143.08	135.58	127.73	119.79	111.96	104.36	97.06	90.08	83.43
6.2	184.42	184.54	183.26	180.84	177.61	173.84	169.69	165.21	160.37	155.08	149.26	142.91	136.08	128.91	121.58	114.25	107.05	100.06	93.34	86.89	80.72
6.4	190.33	189.56	187.00	183.07	178.22	172.82	167.10	161.19	155.11	148.86	142.40	135.75	128.93	122.02	115.13	108.33	101.70	95.30	89.13	83.21	77.52
6.6	194.94	193.31	189.52	184.16	177.83	170.98	163.90	156.77	149.67	142.62	135.63	128.72	121.89	115.19	108.65	102.31	96.19	90.31	84.66	79.23	74.01

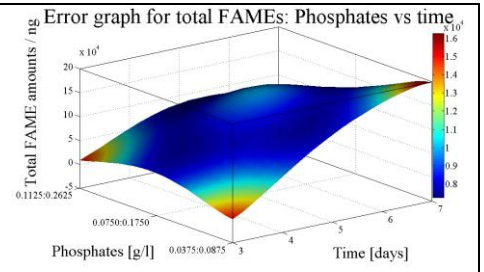
6.8	197.66	195.24	190.39	183.83	176.29	168.28	160.13	152.06	144.15	136.49	129.09	121.97	115.14	108.61	102.36	96.40	90.72	85.30	80.11	75.13	70.34
7	197.80	194.78	189.18	181.83	173.47	164.68	155.81	147.08	138.63	130.54	122.87	115.63	108.81	102.42	96.42	90.78	85.46	80.43	75.65	71.07	66.66

Table 75 - Error graphs for total FAME content derived from algal cells (Nitrates – $NaNO_3$, phosphates – K_2HPO_4:KH_2PO_4, iron – $FeSO_4.7H_2O$, time – days after inoculation) with key to percentage error (red high error, blue low error) (Figure 3-16)




Error graph for total FAMEs: Iron vs phosphates
Total FAME amounts / ng
x 10^5
2
1.5
1
0.5
0
7.47
4.98
2.49
Iron [mg/l]
0.0375:0.0875
0.0750:0.1750
0.1125:0.2625
Phosphates [g/l]
x 10^4
1.6
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8

e


Error graph for total FAMEs: Phosphates vs time
Total FAME amounts / ng
x 10^4
20
15
10
5
0
-5
0.1125:0.2625
0.0750:0.1750
0.0375:0.0875
Phosphates [g/l]
3
4
5
6
7
Time [days]
x 10^4
1.6
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8

f

Table 76 - Design of experiment values used for statistical work a (Figures 3-6 – 3-17)

	2.1	**2.2**	**2.3**	**2.4**	**2.5**	**2.6**	**2.7**	**2.8**	**2.9**	**2.10**
Component	**ng in total algae sample**									
C16:4	5045.71	4218.64	1510.03	963.99	463.43	5122.35	359.17	642.62	370.86	730.81
Oleic acid methyl ester (C18:1n9c)	20371.13	20173.22	2915.15	5165.03	1091.30	36161.44	2325.41	3116.63	2614.87	29939.55
TOTAL	98143.40	90503.84	17240.81	25763.62	9540.99	198460.11	12069.90	41433.29	11744.88	57365.09
saturated FAMEs	37330.83	47107.27	4968.03	11063.93	3468.14	99638.34	3486.29	31593.39	3369.06	8333.10
Monounsaturated FAMEs	23250.96	20560.10	2915.15	5823.05	1632.93	44919.97	3700.65	3599.90	4047.22	31209.14
Polyunsaturated FAMEs	37561.61	22836.47	9357.63	8876.64	4439.92	53901.80	4882.97	6240.00	4328.61	17822.86
%s	**% of total sample**									
C16:4	5.14	4.66	8.76	3.74	4.86	2.58	2.98	1.55	3.16	1.27
Oleic acid methyl ester (C18:1n9c)	20.76	22.29	16.91	20.05	11.44	18.22	19.27	7.52	22.26	52.19
saturated FAMEs	38.04	52.05	28.82	42.94	36.35	50.21	28.88	76.25	28.69	14.53
Monounsaturated FAMEs	23.69	22.72	16.91	22.60	17.11	22.63	30.66	8.69	34.46	54.40
Polyunsaturated FAMEs	38.27	25.23	54.28	34.45	46.54	27.16	40.46	15.06	36.86	31.07

Table 77 - Design of experiment values used for statistical work b (Figures 3-6 – 3-17)

	2.11	**2.12**	**2.13**	**2.14**	**2.15**	**2.16**	**2.17**	**2.18**	**2.19**	**2.20**
Component	**ng in total algae sample**									
C16:4	357.32	353.95	2293.86	0.00	1614.74	1537.82	856.98	295.56	7579.02	980.22
Oleic acid methyl ester (C18:1n9c)	15968.03	10587.77	36322.62	3657.25	23415.46	9656.60	9503.41	20265.99	41598.95	65176.24
TOTAL	26764.53	18934.14	106526.02	10323.00	82061.27	32354.20	18710.60	53117.40	157340.11	157592.68
saturated FAMEs	3108.82	2351.69	28873.20	3724.19	25300.08	6960.97	3120.36	14358.91	42797.93	47136.17
Monounsaturated FAMEs	16754.35	11085.06	43018.96	4791.39	31510.59	11681.88	9996.31	26169.19	51118.84	75219.17
Polyunsaturated FAMEs	6901.36	5497.38	34633.87	1807.42	25250.60	13711.35	5593.93	12589.30	63423.34	35237.33
%s	**% of total sample**									
C16:4	1.34	1.87	2.15	0.00	1.97	4.75	4.58	0.56	4.82	0.62
Oleic acid methyl ester (C18:1n9c)	59.66	55.92	34.10	35.43	28.53	29.85	50.79	38.15	26.44	41.36
saturated FAMEs	11.62	12.42	27.10	36.08	30.83	21.51	16.68	27.03	27.20	29.91
Monounsaturated FAMEs	62.60	58.55	40.38	46.41	38.40	36.11	53.43	49.27	32.49	47.73
Polyunsaturated FAMEs	25.79	29.03	32.51	17.51	30.77	42.38	29.90	23.70	40.31	22.36

Table 78 - Design of experiment values used for statistical work c (Figures 3-6 – 3-17)

	2.21	2.22	2.23	2.24	BBS1	BBS3	BBS4
Component	**ng in total algae sample**						
C16:4	0.00	9317.43	249.61	3643.01	3210.07	8704.54	5957.31
Oleic acid methyl ester (C18:1n9c)	5307.20	10726.22	3447.25	29188.06	57891.19	22751.82	40321.50
TOTAL	16872.67	93189.61	14410.16	141972.70	196271.05	146258.73	171264.89
saturated FAMEs	3734.50	46815.14	5400.06	63435.76	92164.33	64598.47	78381.40
Monounsaturated FAMEs	6452.13	15086.76	3789.38	39231.44	64684.14	31952.93	48318.54
Polyunsaturated FAMEs	6686.03	31287.71	5220.73	39305.49	39422.58	49707.33	44564.95
%s	**% of total sample**						
C16:4	0.00	10.00	1.73	2.57	1.64	5.95	3.48
Oleic acid methyl ester (C18:1n9c)	31.45	11.51	23.92	20.56	29.50	15.56	23.54
saturated FAMEs	22.13	50.24	37.47	44.68	46.96	44.17	45.77
Monounsaturated FAMEs	38.24	16.19	26.30	27.63	32.96	21.85	28.21
Polyunsaturated FAMEs	39.63	33.57	36.23	27.69	20.09	33.99	26.02

Table 79 - Data for optimised C18(1) growth with *Chlorella emersonii* over time a (Figure 3-18)

	day 4	day 5	day 6	day 7	day 8	day 9	day 10
Component	ng in total sample						
Myristic acid methyl ester (C14:0)	1766.57	2099.50	0.00	3170.35	16506.80	16966.40	20769.80
Pentadecanoic acid methyl ester (C15:0)	908.44	0.00	0.00	0.00	7702.96	9687.20	12257.50
Palmitic acid methyl ester (C16:0)	55521.70	63189.40	94044.70	258502.00	1642696.00	1952637.00	1275723.00
Palmitoleic acid methyl ester (C16:1)	0.00	0.00	5934.54	29704.70	201682.00	229478.00	160506.00
C16:2	0.00	0.00	0.00	15675.60	25531.80	67182.40	112560.00
C16:3	0.00	0.00	0.00	49858.90	28473.10	59446.80	144318.00
C16:4	0.00	0.00	6886.22	169900.00	61181.60	106372.00	335180.00
Heptadecanoic acid methyl ester (C17:0)	2468.03	0.00	0.00	0.00	6768.02	31484.80	0.00
cis-10-Heptadecanoic acid methyl ester (C17:1)	0.00	0.00	0.00	0.00	11858.00	54135.50	0.00
Stearic acid methyl ester (C18:0)	30704.50	21714.50	14137.80	21242.60	175384.00	255803.00	156101.00
Oleic acid methyl ester (C18:1n9c)	32904.40	31692.40	135686.00	146459.00	1755404.00	2367454.00	2176059.00
Linoleic acid methyl ester (C18:2n6c)	8640.06	8966.67	14087.30	115036.00	249621.00	459919.00	567804.00
γ-Linolenic acid methyl ester (C18:3n6)	0.00	0.00	0.00	0.00	15264.20	27796.00	39000.30
Linolenic acid methyl ester (C18:3n3)	0.00	29337.40	25678.80	414570.00	280636.00	330628.00	920698.00
C18:4	0.00	3871.88	0.00	49272.40	70443.30	107004.00	146658.00
cis-11-Eicosenoic acid methyl ester (C20:1)	0.00	0.00	0.00	0.00	55804.60	77040.50	68757.90

Table 80 - Data for optimised C18(1) growth with *Chlorella emersonii* over time b (Figure 3-18)

	day 11	day 12	day 13	day 14
Component	ng in total sample			
Myristic acid methyl ester (C14:0)	0.00	0.00	0.00	0.00
Pentadecanoic acid methyl ester (C15:0)	0.00	0.00	0.00	0.00
Palmitic acid methyl ester (C16:0)	1272562.00	1491519.00	1039919.00	0.00
Palmitoleic acid methyl ester (C16:1)	176514.00	159297.00	116854.00	0.00
C16:2	83008.10	99369.00	69278.60	0.00
C16:3	82035.70	113396.00	82894.70	0.00
C16:4	179717.00	235688.00	170510.00	0.00
Heptadecanoic acid methyl ester (C17:0)	8345.79	15478.70	9978.61	0.00
cis-10-Heptadecanoic acid methyl ester (C17:1)	8586.22	12835.70	7976.38	0.00
Stearic acid methyl ester (C18:0)	146066.00	100298.00	126074.00	0.00
Oleic acid methyl ester (C18:1n9c)	2049301.00	1526230.00	1630138.00	0.00
Linoleic acid methyl ester (C18:2n6c)	493477.00	559247.00	378047.00	0.00
γ-Linolenic acid methyl ester (C18:3n6)	39381.80	48039.20	32940.80	0.00
Linolenic acid methyl ester (C18:3n3)	464508.00	595601.00	426108.00	0.00
C18:4	110619.00	139467.00	0.00	0.00
cis-11-Eicosenoic acid methyl ester (C20:1)	58148.90	68272.80	51502.90	0.00

Table 81 - Mixotrophic data (Figure 3-21)

	glucose 3	glucose 2	glucose 5	ethyl acetate	control	glycine	glycerol	spent algae	spent algae re-extracted
Component	**ng in total sample**								
C12:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	36000.00
C14:0	3000.00	3000.00	3000.00	24000.00	7000.00	14000.00	11000.00	40000.00	29000.00
C15:0	4000.00	5000.00	4000.00	3000.00	3000.00	35000.00	2000.00	86000.00	17000.00
C16:0	119000.00	98000.00	78000.00	1762000.00	1129000.00	674000.00	483000.00	3640000.00	3034000.00
C16:1	63000.00	61000.00	45000.00	87000.00	98000.00	232000.00	48000.00	76000.00	177000.00
C16:2	0.00	0.00	0.00	6000.00	15000.00	12000.00	0.00	0.00	502000.00
C16:3	8000.00	8000.00	6000.00	0.00	6000.00	12000.00	0.00	0.00	1098000.00
C16:4	7000.00	11000.00	0.00	0.00	113000.00	243000.00	0.00	7000.00	0.00
C17:0	0.00	0.00	0.00	16000.00	9000.00	6000.00	6000.00	23000.00	0.00
C17:1	0.00	0.00	0.00	5000.00	10000.00	16000.00	0.00	25000.00	0.00
C17:3	0.00	0.00	0.00	0.00	0.00	39000.00	8000.00	0.00	0.00
C17:4	0.00	0.00	0.00	0.00	0.00	0.00	61000.00	0.00	0.00
C18:0	41000.00	40000.00	23000.00	205000.00	147000.00	47000.00	35000.00	428000.00	329000.00
C18:1n9c	207000.00	184000.00	158000.00	583000.00	1414000.00	506000.00	142000.00	212000.00	4953000.00
C18:2n6c	15000.00	13000.00	13000.00	31000.00	200000.00	182000.00	34000.00	0.00	1559000.00
C18:3n6	0.00	0.00	0.00	0.00	16000.00	29000.00	0.00	0.00	0.00
C18:3n3	37000.00	22000.00	24000.00	20000.00	366000.00	816000.00	88000.00	0.00	2848000.00
C18:4	0.00	0.00	0.00	57000.00	87000.00	115000.00	19000.00	196000.00	0.00
C20:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	14000.00	0.00
C20:2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	309000.00	0.00
Total	**502000.00**	**445000.00**	**355000.00**	**2799000.00**	**3621000.00**	**2977000.00**	**938000.00**	**5056000.00**	**14582000.00**

Table 82 - Microwave data (Figure 3-23 and Figure 3-24)

	AP1	AP2	AP3	AP4	AP5	AP6	AP7	AP8	AP9	AP10	AP11	AP12
Component	**ng in total sample**											
C14:0	11800.00	11600.00	12700.00	9780.00	9250.00	8330.00	13600.00	10800.00	15500.00	13300.00	12000.00	17700.00
C15:0	2380.00	2420.00	2550.00	2000.00	1730.00	1810.00	2890.00	2270.00	3630.00	3370.00	2560.00	2890.00
C16:0	1118000.00	1099000.00	1184000.00	839000.00	892000.00	776000.00	1255000.00	968000.00	1266000.00	1035000.00	958000.00	1390000.00
C16:1	207000.00	205000.00	226000.00	177000.00	172000.00	142000.00	242000.00	187000.00	257000.00	211000.00	193000.00	278000.00
C16:2	132000.00	127000.00	139000.00	103000.00	109000.00	94100.00	156000.00	113000.00	155000.00	128000.00	118000.00	167000.00
C16:3	188000.00	179000.00	194000.00	143000.00	142000.00	122000.00	201000.00	157000.00	213000.00	178000.00	165000.00	230000.00
C16:4	130000.00	126000.00	139000.00	108000.00	99600.00	86300.00	142000.00	111000.00	151000.00	126000.00	117000.00	161000.00
C18:0	87500.00	84900.00	92100.00	69900.00	69200.00	60200.00	102000.00	75700.00	115000.00	88300.00	80600.00	118000.00
C18:1n9t	1144000.00	1119000.00	1235000.00	914000.00	899000.00	774000.00	1312000.00	1006000.00	1437000.00	1147000.00	1058000.00	1521000.00
C18:1n9c	69400.00	67500.00	71300.00	52000.00	55200.00	47300.00	73900.00	57400.00	79100.00	64200.00	59900.00	83800.00
C18:2n6c	358000.00	342000.00	370000.00	266000.00	275000.00	235000.00	386000.00	297000.00	418000.00	335000.00	310000.00	446000.00
C18:3n6	1118000.00	1054000.00	1122000.00	794000.00	846000.00	720000.00	1149000.00	893000.00	187000.00	1001000.00	930000.00	1317000.00
C18:4	579000.00	69700.00	75300.00	56100.00	55600.00	47400.00	79200.00	61300.00	86100.00	70800.00	66200.00	93300.00
C20:1	28400.00	28000.00	30700.00	22100.00	22600.00	19400.00	32400.00	24700.00	35500.00	27700.00	25500.00	37000.00
TOTAL	5175000.00	4516000.00	4892000.00	3556000.00	3648000.00	3135000.00	5147000.00	3965000.00	4419000.00	4428000.00	4096000.00	5861000.00

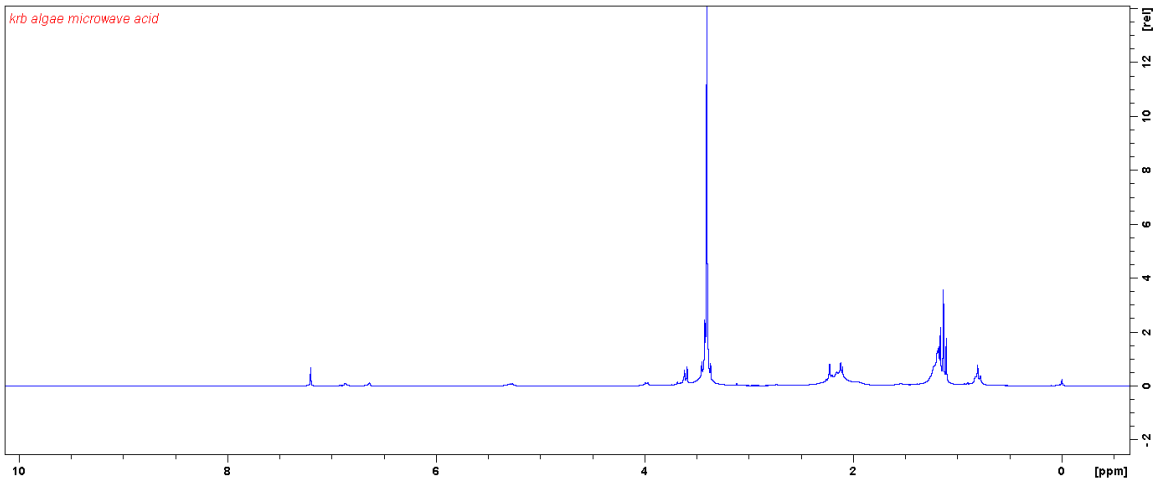


Figure 3 - NMR data from *Chlorella emersonii* microwave transesterification with H_2SO_4

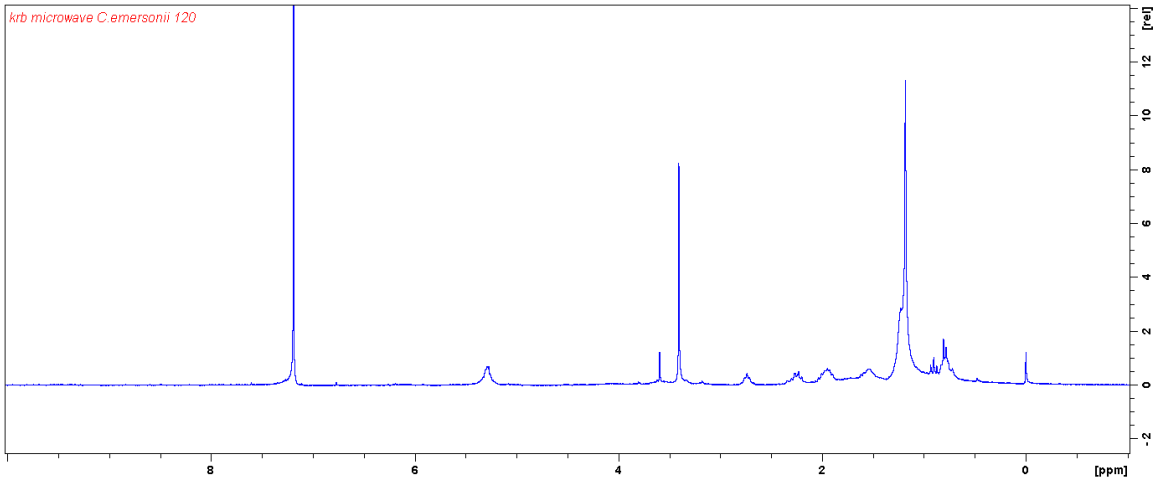


Figure 4 - NMR data from *Chlorella emersonii* microwave transesterification without acid

Table 83 - Comparison of extraction techniques data (Figure 3-25)

	Microwave	Sonication	Soxhlet
Component	**ng in total sample**		
C10:0	38048.20	34912.80	236975.00
C16:0	2151544.00	1339351.00	1825197.00
C16:1	109901.00	37308.70	0.00
C16:4	126376.00	22642.50	0.00
C18:0	365903.00	378144.00	305049.00
C18:1n9c	2060086.00	1561421.00	895111.00
C18:2n6c	291978.00	358849.00	138536.00
C18:3n3	367954.00	312699.00	367102.00
C18:4	110585.00	0.00	0.00
TOTAL	**5622377.00**	**4045328.00**	**3767972.00**